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Investigations of a calcium efficiency trait in cauliflower cultivars /

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INVESTIGATIONS OF
A CALCIUM EFFICIENCY TRAIT
IN CAULIFLOWER CULTIVARS

A Thesis Presented

by

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DEDICATION

This thesis is dedicated to Suzanne, my friend and partner in life. May I someday be as patient and understanding as she.

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CHAPTER 1

LITERATURE REVIEW

The domestication and systematic improvement of crops is the basis of past and present agriculture. Although plant breeding has been a continuous empirical activity since agriculture first evolved, its scientific basis can be traced to Gregor Mendel's classic 1865 paper on the inheritance of seven characteristics in the garden pea, rediscovered in the early twentith century.

Today, breeding efforts are focused on developing more biologically efficient crops adapted to adverse biotic and abiotic conditions due to the increasing costs of energy associated with crop production. Screening crops for variations in nutrient requirements has been an active area of research since Mooer (1922) reported yield differences in cultivars of maize grown on nutritionally poor soil. Primarily, macronutrient-efficient varieties have been sought after because of the immediate economic benefits associated with them, however, micronutrient screening has been gaining in popularity as the list of associated nutritional disorders grows and their influence on crop losses becomes increasingly apparent.

The importance of Ca nutrition has been gaining credence by researchers over the past decade as our understanding and awareness of Ca's role in plant growth and

development expands and becomes increasingly complex.

Because crops have a low requirement for Ca (Wallace and Soufi, 1975; Wiersum, 1979), liming has always been the accepted agricultural practice to supply adequate amounts of Ca to the plant. However, Ca disorders are frequently seen under conditions where adequate Ca concentrations can be found in the soil and insufficient distribution of Ca within the plant has been implicated as the cause for the majority of Ca disorders. This hypothesis is supported by the varying Ca concentrations found in plant parts (Mix, 1976; Marschner, 1974; Maynard, 1979) and the inability of Ca to be translocated to areas where it is required (Lonergan and Snowball, 1969; Wiersum, 1979; Marschner et al., 1974; Kawaski and Moritsugu, 1979). Some nutritional disorders associated with insufficient Ca are tipburn of lettuce (Thibodeau et al., 1969), bitterpit of apple (Delong, 1936; Drake et al., 1966), cavity spot of parsnip (Guba et al., 1961), blossom-end rot of tomatoes (Evans et al., 1953; Estabrooks et al., 1972), tomato cracking (Dickinson et al., 1973), internal browning of brussels spouts (Maynard et al., 1972; Millikan et al., 1966), leaf tipburn in cauliflower (Maynard et al., 1981), and cabbage (Maynard et al., 1965; Palzkill et al., 1976), blackheart of celery (Geraldson, 1954), cavity spot of carrot (Maynard et al., 1961), corkspot in pears (Woodbridge, 1971), leaf tipburn in strawberry (Mason et

al., 1974), and rachis necrosis in selected cultivars of grapes (Cline, 1987). Shear (1975) listed 35 Ca disorders and reviewed factors associated with them. Maynard (1979) reviewed the nutritional disorders of vegetable crops with special emphasis on Ca. Bangerth (1979) also reviewed Ca related physiological disorders and concentrated on Ca uptake, transport, and function in relation to disease. Ferguson (1990) stated that two important nutritional conclusions can be drawn from past Ca studies. These are: 1) Ca is distributed primarily with waterflow along apoplastic pathways, and 2) carefully regulated cellular distribution of Ca is the key to its physiological function. In a comprehensive review article by Hanson (1984), Ca was foreseen as a secondary messenger within the plant. Therefore, Ca must be strictly regulated in order to ensure normal plant functions. The plant's stringent regulation of Ca transport and distribution appears to be the causal agent which leads to insufficient Ca concentrations in certain plant tissues.

Ferguson et al.(1988) partitioned Ca deficiencies into two groups: first, the direct effects that result from inadequate Ca concentrations or a Ca imbalance occurring from antagonistic cations, and second, the existence of tissues that are predisposed to dysfunction when Ca concentrations are low. The former would occur immediately when sufficient Ca concentrations are not present.

For the latter condition, these researchers contend that Ca deficiency sites may exist in tissue, but they manifest themselves only under certain physiological conditions. In this scenario, pools of Ca, that serve as reserves and allow for normal metabolic functioning, fall below a critical concentration required for one or more sequential, cellular activities before a disorder, such as bitter pit of apple, can occur.

Most primary symptoms associated with Ca deficiency are generally believed to be directly related to the role of Ca in maintaining membrane integrity and compartmentalization of organelles within cells (Marschner and Gunther, 1964; Hecht-Buchholz, 1979; Christiansen and Foy, 1979; Atkinson et al., 1980; Bangerth, 1973; Shear, 1975). While studying cytological disorders induced by Ca deficiency in barley, Marschner and Gunther (1964) reported that Ca deficiency results in the breakdown of the tonoplast of vacuolated cells followed by the decompartmentalization of organelles. In potato bud cells, invagination preceded disintegration of the plasmalemma. Breakdown of the plasmlemma was followed by the accumulation of degenerative vesicles and the breakdown of the mitocondria (Hecht-Buchholz,1979).

Microscopic studies have shown initial Ca deficiency symptoms to be a swelling of the cell walls as well as the accumulation of brown substances in the vacuole, intracellular spaces, and the cell wall itself

(Bussler, 1963; Hecht-Buchholz, 1979; Simons and Chu, 1980; Hallam et al., 1973). Some theories have been developed to explain the appearance of these substances including denatured proteins leaked from the cytoplasm (Hallam et al., 1973) and autolysis of cells resulting from increased membrane pore size (Christiansen and Foy, 1979; Garrard and Humphreys, 1967; Williams, 1976). Hecht-Buchholz (1979) stated that Ca deficiency does not appear to affect any particular part of metabolism, but causes autolysis because of a decrease in the membrane integrity. This same researcher notes that many of these symptoms are similar for senescent cells. Christiansen and Foy (1979) also suggested that many Ca deficiency disorders in plants are due to poor membrane function in metabolic compartmentalization. Fuller (1976; 1980) showed that plant tissue containing low Ca maintained a high degree of disorganized cellular arrangement, but no disruption in the cell wall or middle lamella was noted in this study or others (Bangerth, 1973; Hecht-Buchholz, 1979). Even when autolysis has resulted from severe Ca deficiency in potato sprouts, cell walls showed dense lines considered to be Ca pectate and representative of the middle lamella (Hecht-Buchholz, 1979). Although many Ca deficiency disorders can result from a weakening of the cell wall and the middle lamella, ie. water core, bitter pit, blossom-end rot, and tipburn (Atkinson et al., 1980; Bangerth, 1973; Shear, 1975; 1979), these results

would indicate that the cell wall breakdown and the disruption of the Ca pectate are not the causal agent. Bangerth (1979) noted that the mechanisms for these effects of Ca deficiency appear to be less specific than the effects Ca has on membranes.

Sprague (1964) characterized foliar Ca deficiency symptoms as marginal chlorosis, blackening, curling, and necrosis of the apical leaves. Kawaski and Moritsugu (1979) reported that Ca deficient leaves tend to curl toward the midrib, and the margins are usually serrated. Less severely affected leaves show yellow speckling and serrated breaks with lower or older leaves being darker than normal (Clark, 1982). Other recent reports state similar symptoms for Ca deficient leaves (Ku et al., 1991; Maynard et al., 1981; Maynard, 1971;).

In roots, Ca deficiency manifests itself as blackening of root tips and stunted root development. Jackson (1967) observed a reduction in the number of root tips, root laterals, and root hairs when Ca was limiting. Kawaski and Moritsugu (1979) reported that root systems of Ca deficient plants are generally less fibrous and have stubby, dark brown root tips. This poor development hinders the plants nutrient uptake capacity by reducing the total root area. Retention of nutrients by the plant is also reduced by compromising the integrity of the membranes in the roots which leads to an efflux of nutrients (Hanson, 1960).

Ca in the free space, between the soil solution and the plasmalemma is, for the most part, responsible for stimulating potassium uptake and retention by the plant. This stimulation is referred to as the Viets effect (Viets, 1944) and has been shown to work independently of the cells metabolism (Mengel et al., 1967). Insufficient Ca concentrations in this area, below 10^{-4}M, can also cause an efflux of nutrients (Marschner et al., 1966). In experiments using EDTA, Roland et al.(1968) showed that the ability of Ca to reduce membrane permeability is responsible for the Viets effect. This reduction in permeability is thought to be due primarily to Ca substituting for hydrogen ions and binding negative charges of the plasmalemma with the cell wall, thus decreasing its permeability (Van Steveninck, 1965). Studies by Roland and Bessoles (1968) which revealed that Ca is more prevalent located between the cytoplasm and the cell wall, indicating high Ca concentrations in the plasmalemma, support Van Steveninck's work. Poovaiah and Leopold (1976) showed that Ca chloride decreased betacyamin sulfate leakage out of membranes but ammonium sulfate increased it, further adding to the evidence that Ca deceases membrane permeability.
Epstein (1961) and Rain et al. (1964) both conclude that Ca is essential for normal transport and retention of ions by the plant. Since net uptake of nutrients by the plant is the difference between influx and efflux, the Viets effect can

be said to increase net uptake although the method by which it does so is by greater retention of nutrients by the plant rather than by influx.

In fruits and storage organs Ca deficiency may appear as water-soaked lesions, such as in watermelon and tomato, and appear on the distal end of the fruit (Maynard, 1957; Waters et al., 1961). Brussels sprouts display an internal browning of the axillary buds (Maynard, 1972). In apples, low Ca concentrations have been correlated with bitter pit of apple and senescent breakdown (Sharples, 1980; Autio et al., 1986). Ca content in apples has been shown to reach a maximum level early in fruit development and concentrations decline as the fruit expands (Ferguson et al., 1989). This change is thought to be due to nutrients and water being supplied to the expanding fruit primarily through the phloem, since magnesium and potassium concentrations do not decline (Ferguson et al., 1989). Large quantities of Ca would be excluded from entering the fruit at this stage since it is transported, for the most part, via the xylem (Maynard, 1979). Bramlage et al. (1990) and Kubowicz et al. (1982) suggested that seeds enhance the translocation of Ca into the fruit via auxin synthesis.

Research such as that of Bramlage et al. (1990) and Kubowicz et al. (1982) helps to underline the complexity involved when explaining Ca movement into fruit. In cauliflower the marketable portion, the curd, usually does

not exhibit Ca deficiency symptoms in the field except under severe conditions. Usually it appears as tipburn on the young, actively growing leaves, similar to cabbage and lettuce (Maynard et al., 1979; 1981). After harvest however, cauliflower quickly develops brown curd, as a result of Ca deficiency, rendering it unmarketable.

Although auxins themselves have been shown to decrease Ca movement (Bangerth, 1973; Oberly, 1973; Stahly and Benson, 1976; Wieneke et al., 1971), the acropetal transport of Ca and the basipetal transport of auxin have been shown to be closely associated (Banuelos et al., 1987; 1988; De Guzman et al., 1984; Hepler et al., 1985; Lee et al., 1984). Work using IAA (auxin) and TIBA (a known inhibitor of basipetal auxin transport) injected into peppers has been shown to increase and decrease Ca accumulation respectively (Marcelle et al., 1981). Similar results were reported by other researchers treating other plants with TIBA (Dela Fuente and Leopold, 1973 Marschner and Ossenberg-Neuhaus,1977; Bangerth,1976). One hypothesis that explains the auxin-Ca relationship is that auxins increase the mobility of Ca in the xylem and the phloem (Bangerth, 1976). Marschner and Ossenberg-Neuhaus (1979) suggested however, that auxins increase the number of binding sites in the apoplast which results in an increase in Ca uptake. Knight and Crooke (1973) found a simultaneous increase in the cation exchange capacity and Ca content from

the stigma to the ovary in the flower Antirrhinum. Earlier, pollen was reported to show a chemotropic response to Ca for the same species (Masarenhaus and Machlis, 1964). Additionally, increased lateral distribution of Ca has been reported for IAA-stimulated growth (Goswami and Audus, 1976). De Guzman and DeLa Fuente (1984) suggested that an increase in the concentration of charged auxin in the cytoplasm causes a depolarization in the membrane potential. Ca responds to this depolarization by flowing into the cell and activating a hormone carrier that transports auxin to the cell wall. Earlier, Goldsmith (1977) put forth the chemiosmotic hypothesis of IAA-transport which explained the IAA-Ca relationship through the existence of these Ca dependent IAA carriers. Other research has shown that IAA's entry into the cell and its binding to a carrier are very site specific (Goldsmith, 1982; Jacobs and Gilbert, 1983). Barney (1987), working with sunflower hypocotyl segments, concluded that basipetal IAA transport resulted in a decrease in Ca found in the free space. Earlier, Drobak and Ferguson (1985) provided evidence that the free space was a major source for Ca influx to the cytoplasm. In another study using protoplasts from etiolated soybean hypocotyls, Cohen and Lilly (1984) reported a decrease in CA uptake and an increase in the efflux of radioactive Ca when protoplasts were cultured with active auxins.

Similar results were not seen when protoplasts were cultured

with antiauxins. Cohen and Lilly (1984) suggested that auxin lowers Ca ion concentrations by changing Ca flux at the plasma membrane. This theory is supported by research that shows that auxin elevates Ca ATPase activity, which would allow for changes in Ca concentrations to occur (Kubowicz et al., 1982). This explanation relates well to cytoplasmic steaming, since low Ca concentrations have been shown to promote streaming (Kamiya,1981) and earlier studies showed that auxin promotes cytoplasmic streaming (Thimann and Sweeney, 1937). Ca-auxin relationships have been implicated as the cause for some Ca deficiency disorders. Tipburn in lettuce has be associated with higher than normal levels of auxin. Chlorogenic acid inactivates IAA oxidase and will normally increase when there is insufficient Ca. Lettuce cultivars that showed a tolerance to tipburn had higher levels of chlorogenic acid than the nontolerant cultivars (Collier et al., 1979). High levels of chlorogenic acid would allow for high levels of auxin due to the inactivation of auxin oxidase. These high levels of auxin oxidase would reduce Ca translocation which could lead to tipburn. However, Bangerth (1979) suggested that the acidifying effects of Ca on auxin-treated tissue and auxin binding were not associated with Ca deficiency disorders.

In contrast to auxin, cytokinins have been shown to increase Ca translocation to the upper portions of the plant (Shear and Foster, 1970) and to enhance Ca uptake in mung

bean hypocotyls (Lau and Yang, 1975). Bangerth (1979) suggested that cytokinin increases Ca movement because senescence is retarded. Isermann (1970) states that this is due to an increased transpiration rate which enhances Ca influx. Higher rates of Ca uptake were attributed to a modification of the phosphorylation reactions of membrane proteins (Ralph et al., 1976). Leopold et al. (1974) reported that the cytokinin benzyladenine and added Ca increased lateral shoot development in soybeans. Ammonium has been shown to inhibit shoot development and to enhance senescence in maize (Poovaiah and Leopold, 1973a). These same researchers reported that Ca canceled out the effects of ammonium. There is substantial evidence that Ca plays an important role in mediating bud development initiated by cytokinin. While working with Funaria moss, Saunders and Hepler (1981) showed that cytokinin increases membrane associated Ca in the region of cells undergoing differentiation to become buds. Similar results were found when protonemata were cultured in ionophore A 23187 and Ca was supplied in the absence of cytokinin (Saunders and Hepler, 1982). When EDTA was used to chelate Ca and reduce the extracellular Ca in conjunction with Ca transport blockers, bud initiation was inhibited (Saunders and Hepler, 1983). In these same articles it was pointed out that the initial buds do not develop into complete buds and that cytokinin must initiate or provide other stimuli in addition

to those that are Ca dependent in order for proper bud development.

Ca transport to the shoot apex and fruit are directly depressed by gibberellins (Wieneke et al., 1971; Bangerth, 1973; Wills et al., 1975). Senescence and abscission have been shown to be promoted by the combination of gibberellic acid and ammonium and inhibited by gibberellic acid and Ca (Poovaiah and Leopold, 1973a; 1973b). These same authors also reported that Ca can reverse the effects of ammonium. Leopold (1977) suggested that Ca accomplishes this effect by altering the affinity of the attachment site for gibberellic acid. Other reports have shown that gibberellic acid-stimulated growth is enhanced by Ca (Leopold, 1977; Leopold et al., 1974). A lot of interest has been shown for the Ca-GA relationship since Chrispeels and Varner (1967) first reported Ca's involvement in alph-amylase secretion. Since then, studies using inhibitors and low temperatures have shown that Ca regulates secretion at the plasma membrane rather than the cell wall (Moll and Jones, 1982; Mitsui et al., 1984), and different isozymes of alph-amylase, which react differently to GA and Ca have been identified (Jones and Jacobsen, 1983; Jones and Carbonell, 1984). While isozyme 2 secretion is independent of GA and Ca, isozymes 3 and 4 requires both, and isozyme 1 requires only GA. More recently, Bush et al. (1989) reported that the endoplasmic recticulum is the principle site of Ca transport in barley

aleurone cells and gibberellins can stimulate the Ca transport rate several fold. This supports existing evidence that the endoplasmic recticulum and vacuoles can accumulate Ca concentrations above those found in the cytoplasm by utilizing ATP-driven pumps located on their membranes (Poovaiah and Reddy, 1987; Schumaker and Sze, 1987; Bush and Sze, 1986; Giannini et al., 1987). It has been speculated by these researchers that Ca stored in organelles is used to modulate cystolic Ca levels. The relationship between Ca and gibberellic acid is not always protagonistic. This relationship is clearly seen when examining GA's ability to enhance cell elongation, a process that is inhibited by Ca (Tagawa and Bonner, 1957; Moll and Jones, 1982). Moll and Jones (1982) suggested that GA promotes Ca uptake and release from the cell wall to permit growth to occur.

Very little has been reported about Ca and ethylene interactions even though ethylene is able to mimic some Ca deficiency symptoms. Suttle and Kende (1978) report that ethylene increases membrane permeability and respiration, and hastens senescence and fruit ripening. Ca has been shown to reduce respiration and suppress ethylene production when it is added to Ca deficient tissue (Faust, 1975; Poovaiah and Leopold, 1973). The benefical effects of Ca are attributed to Ca maintaining membrane integrity. Cell walls have been identified as the site for initial Ca

deficiency symptoms to appear (Hecht-Buchholz, 1979); while one site of ethylene synthesis in apples has been associated with cell wall membranes (Mattoo and Lieberman, 1977; Lieberman and Wang, 1982). Ca has also been shown to prevent the expression of some ethylene responses in peas (Leopold et al., 1974) and cucumber cotyledons (Ferguson et al., 1983). These findings suggest that ethylene may be responsible for the expression of some Ca deficiency symptoms and that Ca prevents these ethylene responses when internal Ca concentrations are adequate.

Other areas of research involving Ca-hormonal interactions include pollen tube growth, self-incompatability, and the transfer of information between pollen grain and pistil. The specific role Ca plays in each of these areas is not fully understood (Bednarska, 1989; Polya et al., 1986; Singh and Paolillo Jr., 1990; Kauss, 1987).

Much of the initial work done on mineral nutrition emphasized ecotypic adaption of wild species to vaying soil conditions and their tolerance to heavy metals. The recognition of species of plants, adapted to calcareous soils, led to the categorizing of calcicole-calcifuge species (Rorison, 1960; Snaydon, 1962; Jefferies and Willis, 1964; Clarkson, 1965; 1966; 1967). Identification of independent effects on plant growth has been difficult because of the many interrelated changes associated with

limestone and acid soils. However, soil pH (Rorison, 1960), and Ca concentration (Rorison, 1960; Snaydon, 1962; Clarkson, 1965) have been documented as two factors contributing to limited plant growth on calcicole and calcifuge soils. Plants that are able to grow and reproduce successfully under these conditions have developed yarious survival mechanisms. One such mechanism is described by Grime and Hodgson (1968) who found tolerance to low pH was associated with tolerance to high levels of available aluminum. This tolerance was linked to the formation of an aluminum chelating compound in the root.

The ability of Ca to counteract the detrimental effects hydrogen ions have on membranes may be partially responsible for Ca's influence on ion uptake (Jacobson et al., 1960; 1961a; 1961b; Rain et al., 1964; Marschner et al. 1966) reported that Ca had its greatest influence near pH 4.5 indicating a counterbalance to high hydrogen ions. Rains et al. (1964) suggested that hydrogen ions damage membranes directly. Earlier, Fried and Noggle (1958) noted that hydrogen has a competitive effect with the binding of cations to sites on the membrane. The effect of Ca may be to counteract these properties, thus maintaining selectivity and increasing ion uptake. Studies using chelating agents have enhance the concept that Ca maintains membrane integrity and stability.

Many researchers have reported reduced uptake of nitrate,

phosphorus, and other anions when Ca was removed with EDTA (Foote and Hanson, 1964; Hyde, 1966; Legget et al., 1965)

Tolerance to excess hydrogen ions in solution vary widely within and between plant species. Rice roots have been shown to be unaffected by pH levels of 3.5 when aluminum is not present (Thaworuwog and Van Diest, 1974) and yellow poplar tolerated pH levels of 4.0 to 5.5 (Patel and Mugwira, 1974). Islam et al. (1980) showed marked differences in the responses of ginger, cassava, maize, french beans, wheat, and tomato when pH ranges were changed to register outside 5.5 to 6.5. From pH 3.3 to 4.0, tolerance was ranked as follows: ginger > cassava > tomato > french beans > wheat > maize. Earlier, Ciat (1977) reported similar findings with cassava, beans, and maize under acidic field conditions. In four sunfower cultivars, differences in acidic tolerance was reported to be above pH 4.0. Critical pH levels above which yields were increased varied from 4.0 to 5.0. Root yields of the same cultivars showed differences when pH was above 3.5 (Blamey et al., 1982). Maas (1969) reported that H ions in pH 3 to 5 reduced Ca uptake in excised maize roots. Lund (1970) found that soybean roots required a higher Ca level at pH 4.5 than 5.6. Siraj-Ali et al. (1987) reported that optimal growth and nutrient uptake of Chrysanthemum morifolium 'Bright Golden Anne' was achieved at pH 6.5 in both Hoagland's solution and Peter's Hydro-Sol.

Several review articles describing genetic differences in plant nutrition were published in the early 1960s (Myers, 1960; Vose, 1963; Gerloff, 1963; Epstein and Jefferies, 1964). More recently, Vose (1981) published an extensive review dealing with the effects of genotypic factors on plant nutrient requirements. Differences in nutrient accumulation have been shown to be genetically controlled. Ca concentrations in the earleaf of corn have been reported to be under the control of three genes acting in an additive manner (Gorsline et al., 1964; 1968). Naismith et al. (1974) suggested that the genetic loci influencing Ca, phosphorus, and magnesium accumulation in corn is present on chromosome nine. The resistance to blossom end rot, a Ca disorder of tomatoes, has been found to be a recessive trait (Greenleaf and Adams, 1969). Resistance could be based on lower requirements for Ca in the fruit, or greater efficiency with which the plant accumulates Ca in the fruit. It was suggested that there is an association between the genotype for uniform fruit ripening and the high incidence of blossom-end rot (Trinklein and Lambeth, 1976). Longeragan and Snowball (1969) compared the Ca concentrations of eighteen different species of plants grown in solutions with those grown in the field and found little difference in the Ca content of a particular species, whether the plant was grown in soil or solution culture. Myers (1960) reviewed the literature on genotypic variations

within and between species for element content and concentration, and concluded that cultivars showed marked variations in the accumulation of different elements, indicating the presents of independent mechanisms under different genetic control. Vose (1963) showed that differential nutrient uptake and differential tissue requirements for elements are primary forms of nutrient efficiency. The identification of these sites of ion uptake and the understanding of the mechanisms involved with this process are important for a better understanding of the physiological basis of genotypic differences in nutrient requirements.

Cultivar differences in Ca uptake, distribution and susceptibility to Ca deficiency disorders have been reported for many crops. In tomatoes, Giordano et al. (1982) showed efficient utilization and a greater ability to absorb Ca from low Ca solutions was responsible for the differences. Using radioactively labelled Ca, Behling (1987; 1989) showed efficient lines were able to carry Ca into the leaf lamina in the absence of transpiration. In lettuce, Banuelos et al. (1988) showed that basipetal auxin transport still favors acropetal Ca transport under low transpirational conditions. This observation suggests that a possible physiological basis for efficiency could be greater basipetal auxin transport.

Other crops that have shown intraspecific differences in Ca

requirements include peanuts (Beringer et al., 1976; Walker, 1975), lupin (Alva et al., 1990; Hocking et at., 1977), brussels sprouts (Millikan et al., 1966), cabbage (Nieuwhof, 1960), cucumber (Engelker et al., 1990), cowpea (Horst, 1987), cauliflower (Hochmuth, 1984;) , corn (Brown, 1967; Gorsline et al., 1965), soybean (Kleese, 1968), legumes (Andrew et al., 1961), and collards (Johnson, 1991).

The possibility that variations in response to Ca stress exists between strains of cauliflower was first suggested by Maynard et al. (1981). Hochmuth (1984) gave validity to this suggestion by developing a cauliflower screening program for Ca that showed large differences in dry matter accumulation between strains. More screening of existing strains is required however, and physiological and genetic studies are needed for a better understanding of how Ca functions in cauliflower.

The objectives of this study are as follows: 1) screen commercial strains of cauliflower for tolerance to low calcium, 2) classify strains as efficient or inefficient, 3) study the effects different external pH regimes will have on the calcium efficiency trait in selected strains, 4) investigate the genetic control of calcium efficiency in cauliflower.

CHAPTER 2

RESPONSE OF CAULIFLOWER CULTIVARS TO LOW-CA FIELD CONDITIONS DIFFER FROM RESPONSE IN LOW-CA SOLUTION CULTURE

2.1 Abstract

Twenty-six commercial cultivars of *Brassica oleracea*, botrytis group varied in their ability to tolerate low Ca concentration in solution culture. Significant differences were observed among cultivars for dry matter yields, plant Ca-deficiency symptoms, plant Ca content, and Ca-efficiency ratios (mg of dry matter produced per mg of Ca accumulated). Five cultivars were classified as efficient for Ca accumulation and utilization; seven were moderately efficient, and ten were classified as inefficient. Three strains were efficient at accumulation only and one was efficient at utilization only. In a field study, where soil Ca was low to moderate and pH was 5.3, there were variations among the cultivars for leaf Ca deficiency symptoms and percent marketable curds. Efficient cultivars had slightly fewer Ca deficiency symptoms at early plant growth and symptom expression was more uniform among individual plants than most moderately efficient and inefficient cultivars.

No differences for Ca deficiency symptoms were observed when curds were mature.

There was no relationship between leaf Ca deficiency symptoms in the field and curd marketability. All cultivars classified as Ca-efficient by hydroponic screening produced high percentages (88% to 100%) of marketable curds in the field. Moderately efficient and inefficient cultivars were more variable, yielding high, moderate, and low percentages of marketable curds.

2.2 Introduction

Genetic variability within plant species that allows plants to adapt to adverse environmental conditions is the basis for screening and selecting strains for tolerance to suboptimal nutrient levels (Gerloff and Gabelman, 1983; Vose, 1984). Differences in plant tolerance of low Ca have been reported for corn (Clark, 1978) and legumes (Andrews and Norris, 1961; Kleese, 1968). The basis for low-Ca tolerance in crops has been linked to efficiency in Ca accumulation, transport, or utilization, either singularly or in combination (Brown, 1967; Gorsline et al.,1965; Kleese, 1968; Marschner, 1986).

Giordano et al.(1982) screened 138 tomato strains for tolerance to low Ca and reported that tolerant strains were able to remove more Ca from a low-Ca solution and utilize the Ca more efficiently for dry matter production than non-

tolerant strains. Ca efficiency in one tomato strain was due to better distribution of Ca under low-transpiration conditions (Behling et al., 1989). Inheritance studies by Giordano et al.(1982) and Li and Gabelman (1990) showed additive and dominant gene effects were important to the Ca efficiency trait in tomato. Variations in tolerance to low Ca have also been reported for Brassicacea species. In collards, Johnson (1991a,b) reported efficient cultivars had higher Ca efficiency ratios (CaER) for young leaves than inefficient cultivars. A smaller root system may result in a greater susceptibility to tipburn, a Ca deficiency disorder (Maynard et al., 1981), under moisture stress. However, a smaller root system associated with the inefficient cultivar did not affect Ca accumulation or Ca concentration found in the collard plant (Johnson,1991a,b). Cauliflower strains have also been shown to vary in their ability to tolerate low Ca in solution culture (Hochmuth, 1984). Variations in Ca efficiency were attributed to increased Ca accumulation and utilization. Tipburn in cauliflower has been reported to reduce product quality and marketability when the curds are discolored by Ca deficiency and when damaged tissue is attacked by pathogens (Maynard et al., 1981). Both gypsum, applied to the soil as a Ca source, and Ca applied in foliar sprays were ineffective at alleviating Ca deficiency symptoms in cauliflower (Rosen and Burchite, 1987; Rosen, 1990).

The research with several crops shows that fertilizer management, environment, and genetics play roles in Ca nutrition. Breeding Ca-efficient cultivars may help reduce losses of crops to Ca deficiency under field conditions.

As a first step towards breeding cultivars for tolerance to suboptimal Ca levels, efficient strains must be isolated through screening programs that will yield useful and predictable responses under field conditions. This study was conducted to screen 26 commercial cauliflower cultivars for their ability to tolerate low-Ca solution culture and to evaluate these same cultivars under low-Ca field conditions.

2.3 Materials and Methods

Seeds of twenty-six commercial cultivars of *Brassica oleracea*, Botrytis group were germinated in perlite soaked once with modified Hoagland and Arnon (1950) nutrient solution (0.07 mM KH_2PO_4, 0.15 mM $MgSO_4$, 0.36 mM $NaNO_3$, 3.57 uM KCl, 1.79 uM H_3BO_3, 0.36 uM $MnSO_4$ H_2O, 0.15 uM $ZnSO_4$, 0.04 uM $CuSO_4$, 0.07 uM $(NH_4)_6Mo_7O_{24}$, and 2.86 uM Fe in the form of FeEDTA). Calcium (125 uM) was supplied from $CaCl_2$.

Approximately 18-21 days after sowing, single seedlings were transferred to individual 1.5-liter polyethylene vessels containing 1.4 liters of nutrient solution (5 mM KNO_3, 1 mM KH_2PO_4, 2 mM $MgSO_4$, 5 mM $NaNO_3$, 50 uM KCl, 25 uM H_3BO_3, 5 uM $MnSO_4$ H_2O, 2 uM $ZnSO_4$, 5 uM $CuSO_4$, 1.5 uM

$(NH_4)_6Mo_7O_{24}$ $4H_2O$, and 40 uM Fe as FeEDTA). Calcium (375 uM) was suppied from $CaCl_2$. Each vessel was lined with a black polyethylene sheet to deter algal growth by reducing light penetration. Each seedling was suspended in nutrient solution by supporting the stem of the seedling with a foam plug placed in a hole in the lid of the polyethylene vessel. Filtered air was bubbled continuously through the nutrient solution in each vessel for the duration of the experiment. The original volume of solution was maintained by adding deionized water daily. Light intensity and daylength were supplemented with high-intensity sodium lamps to achieve an irradiance level, two meters from the light source, of 380 umol s^{-1} m^{-2} for a period of twelve hours each day. Twenty-six cultivars were arranged in a randomized complete block experiment (4 replications) and allowed to grow for five weeks in a glasshouse located at the University of Massachusetts, Amherst, MA during the months of April and May in 1984. Temperatures in the glasshouse were set at 24C days and 16C nights. Plants were visually rated for Ca deficiency symptoms, removed from the vessels, and roots were separated from shoots. All plant material was dried at 65C in a forced-air drying oven. Tissue samples were wet-ashed with HNO_3 and H_2O_2, and Ca analyses were carried out by atomic absorption spectrophotometry (Greweling, 1976). Analysis of variance was conducted for dry matter accumulated, visual plant symptom ratings, Ca accumulated,

and Ca efficiency ratios (CaER=mg plant dry matter produced per mg Ca in the plant).

For the field experiment, seeds were germinated in perlite, and seedlings were grown in individual cells containing equal volumes of soil, sand, and peat. Prior to transplanting in the field, plants were placed in cold frames to acclimate seedlings. Field work was conducted in May-July in 1984 at the University of Massachusetts Research Farm in South Deerfield, MA. Soil was a Hadley very-fine sandy loam, variant (Typic Udifluvent, Si, mixed, mesic infrequently flooded). Soil properties to a depth of 15 cm were: organic matter 2g.kg^{-1} (wet digestion); pH 5.3 [1 soil: 1 water (w/v)]; P 18 mg.kg^{-1} (Bray P1); K 98 mg.kg^{-1}; Ca 410 mg.kg^{-1}; Mg 55 mg.kg^{-1} (2N KCl). Cation exchange capacity was 2.2 meq/100 g. Soil calcium for this soil was low to medium according to the Soil and Plant Tissue Testing Lab, West Experimental Station at the University of Massachusetts, Amherst, MA. A fertilizer mixture [10-10-10 (NPK) no Ca] was broadcast at 180 kg/ha and incorporated in the field soil by disking prior to planting.

Twenty-six cultivars were arranged in a randomized complete block experiment with 5 replications. Each plot contained ten plants resulting in a total of fifty plants for each cultivar. Each plant was supplied with 500 ml of a nutrient solution (4 g of 20-8-16 NPK no Ca per liter), and diazinon insecticide when transplanted, and at biweekly

intervals for six weeks. Additional N (28 kg/ha) was applied by side dressing with ammonium nitrate at the same time. Total N applied to the crop was 171 kg/ha. Plants were spaced 51 cm apart within rows with 76 cm between rows (Lorenz and Maynard, 1980). After one week in the field, plants were side-dressed with sodium boron and potassium-magnesium sulfate to achieve 1kg B/ha, 60kg K/ha, 30kg Mg/ha and 45kg S/ha by banding the fertilizer next to the row. Beginning two weeks after transplanting, new plant growth was visually rated 1 to 5, on a weekly basis, for tipburn (1=no symptoms; 2=slight tipburn present on leaf margins; 3=tipburn, necrosis prevalent extending in from leaf margins, curling may be present; 4=Tipburn, necrosis extends to midrib, curling; 5=severe necrosis,leaf development is reduced). Curds were rated marketable or unmarketable depending on the presence of any discoloration (browning and glassy appearance) due to insufficient Ca (Maynard et al., 1981). Nutrient analysis was not conducted on curds. Most cultivars produced 100% marketable curds based on size. Cultivars that developed curds that were less than minimum marketable size (<15 cm in diameter) were noted.

2.4 Results and Discussion

In the solution culture experiment, all cultivars efficient at accumulation removed greater than 50% (>7.5 mg) of the 375 umol Ca from solution.

Cultivars efficient at utilization had CaER values above 500, while moderately efficient cultivars were above 400. All others were inefficient for Ca utilization. Cultivars classified as "E" for efficient, met the criteria for efficiency in accumulation and utilization. Inefficient cultivars "I", did not show efficiency in either category. Moderately efficient cultivars "M", accumulated greater than 7.5 mg of Ca but CaER values fell between 400-500. There were four exceptions, three which were classified as "EA", efficient at accumulation only, and one strain that was classified as "EU", efficient at utilization only (Table 2.1). Those cultivars classified as efficient at accumulation produced very little dry matter but accumulated large amounts of Ca. The cultivar efficient at utilization produced a large amount of dry matter but accumulated very little Ca.

Significant differences were observed among cultivars for accumulated dry matter, Ca content, Ca deficiency symptoms, and calculated CaER (Table 2.1). Plant dry matter yields ranged from 0.55g to 7.50g, a 13.6-fold difference. Root Ca content ranged from 0.28mg to 0.92mg with considerable variability among cultivars within efficient, inefficient, and moderate groups (Data not shown). Total Ca content of plants varied over a 3.75-fold range. Plant Ca deficiency symptoms varied from 1.0 to 4.5 on a scale of 1 to 5, and CaER varied from 190 to 680 (Table 2.1).

These ranges in values for the same variables are similar to those reported by Hochmuth (1984).

Leaf symptom data from the field experiment are presented for the first evaluation (Wk 2) and for the day curds were evaluated. Results from intermediate weeks were not significantly different. Recently matured wrapper leaves of 'Self-Blanche' were removed from plants when curds were evaluated, and analyzed for Ca. Leaves displaying tipburn contained 1.8 $g.kg^{-1}$ Ca at the tips and 4.9 $g.kg^{-1}$ Ca at the base. Leaves without tipburn had 6.6 $g.kg^{-1}$ Ca at the tip and 5.1 $g.kg^{-1}$ Ca at the base. These values are similar to those reported by Maynard et al. (1981), Rosen (1990) and Rosen and Buchite (1987) for Ca-sufficient and Ca-deficient cauliflower leaves. 'White Dove' failed to develop curds, and 'White Contessa' flowered shortly after it was planted in the field. There were significant differences among cultivars for Ca deficiency symptoms and curd marketability (Table 2.2). Inefficient and moderately efficient cultivars developed slightly more severe deficiency symptoms and more variability among plants in Ca deficiency symptoms at week 2 than efficient cultivars (Table 2.2). The cultivar efficient at utilization, 'Snowball E', had significantly fewer Ca deficiency leaf symptoms when curds were evaluated than at week 2 (Table 2.2). Two efficient accumulators, 'White Summer' and 'Supermax RS', were unchanged in Ca deficiency

symptoms. 'Snowball 99' showed a significant reduction in symptoms.

Efficient cultivars, as classified by the solution culture experiment, produced 88% to 100% marketable curds (Table 2.2). Three moderately efficient cultivars produced 86% to 100% marketable curds, and three others produced only 20% to 44% marketable curds. Inefficient cultivars varied dramatically, but, five inefficient cultivars produced 80% to 98% marketable curds. Two of the three cultivars classified as efficient at accumulation produced 100% marketable curds, whereas the third produced only 56% marketable curds. "Snowball E", classified as efficient at utilization, yielded 72% marketable curds.

Visual differences in tipburn were originally reported between 'Imperial 10-6' and 'Self-Blanche' under field conditions and glasshouse experiments (Maynard et al., 1981). Differences between these cultivars were also observed here in both solution culture and early in the field. However, no visual differences between these cultivars for tipburn were observed when curds were fully developed. Rosen (1990) reported the incidence of tipburn was higher in 'Snow Crown' than 'Self-Blanche' or 'Imperial 10-6'. 'Snow Crown' showed one of the largest increases in deficiency symptoms in the field study.

The results from the solution culture studies showed that two aspects of Ca efficiency exist in cauliflower as

originally reported by Hochmuth (1984). Hydroponic screening allowed for cultivars efficient and inefficient at Ca accumulation or Ca utilization to be partitioned effectively.

The varied response of cauliflower cultivars to low-Ca conditions in the field vs. solution culture shows environmental conditions can greatly affect Ca nutrition in cauliflower. Inefficient cultivars improved considerably in their response to low-Ca in the soil compared to solution culture. It is possible that growing conditions in the field, e.g. temperature and moisture were optimal for cauliflower curd production. The soil test results indicated that the soil was low in Ca. However, the unrestricted root volume in the field contained enough total Ca for plant growth and yield for most cauliflower cultivars. Results might be different in soils with lower Ca concentrations. Our results in the field showed that care should be exercised in extrapolating greenhouse screening results to cauliflower performance in the field.

Table 2.1 Response of 26 cauliflower cultivars to low Ca in solution culture.

Cultivar	Source[y]	Dry matter yield (g/plant)	Plant symptoms[x]	Ca content (mg/plant)	CaER	Class[w]
Improved Holland						
Erfurt	1	7.50	1.3	11.3	680	E
Snow Crown	1	7.00	1.0	10.8	640	E
Olympus	7	5.30	1.3	10.0	575	E
White Summer	3	2.40	3.5	10.0	250	EA
Imperial 10-6	2	5.30	1.3	9.7	570	E
Snowball Y	1	4.50	1.3	9.6	460	M
Suprimax RS	5	3.50	2.3	9.1	380	EA
Spring Snow	6	3.75	2.5	9.3	450	M
White Dove	6	4.00	2.0	9.0	430	M
Snowball 99	1	3.40	2.0	9.0	335	EA
Snowball 123	2	3.70	1.5	8.8	480	M
White Contessa	6	5.75	1.8	8.8	650	E
Early Snowball A	8	3.75	2.5	8.5	450	M
Snowflower	7	4.00	1.3	8.1	500	M
White Top	3	3.00	2.0	8.0	400	M
Christmas White	6	1.20	4.5	7.0	190	I
Andes	5	1.50	4.5	6.8	250	I
Snowball E	4	3.68	2.5	6.5	570	EU

Table 2.1 continued

Cultivar	Source[y]	Dry matter yield (g/plant)	Plant symptoms[x]	Ca content (mg/plant)	CaER	Class[w]
Atos	5	2.45	3.0	6.4	355	I
White Fox	3	1.80	3.5	6.3	300	I
Super Snowball	4	0.90	4.5	4.2	230	I
White Rock SG-11	3	1.30	3.5	4.0	300	I
Self-Blanche	2	0.90	4.5	3.9	242	I
Snowball 34	1	0.68	4.0	3.6	210	I
White Empress	4	0.88	3.5	3.4	270	I
Racket	3	0.55	4.5	3.0	235	I
$LSD_{0.05}$		0.9[z]	1.3[z]	1.0[z]	28[z]	

[z]Mean separation within columns by LSD, P=0.05. Data presented are the means of five observations.

[y]Sources: 1=Agway Seed Co.; 2=Harris Seeds; 3=Sluis & Groot; 4=Twilley Seeds; 5=Royal Sluis; 6=T.Sakata & Co.; 7=Asgrow Seeds; 8=Peto Seed Co.Inc.

[x]Symptoms: 1=no symptoms; 2=necrotic lesions; 3=moderate lesions, minor curling of youngest leaves; 4=moderate curling, necrosis; 5=severe curling, necrosis, reduction in growth.

[w]Class: E=efficient; EA=efficient at accumulation only; M=moderately efficient; EU=efficient at utilization only; I=inefficient.

Table 2.2. Response of 26 cauliflower cultivars to low-Ca field conditions.

Cultivar	Visual rating[y] week 2	Visual rating[y] week of curd evaluation	Curd evaluation (% marketable)	Class[x]
Improved Holland				
Erfurt	1.1	1.5	98	E
Snow Crown	1.3	2.7	88	E
Olympus	1.2	1.1	92	E
White Summer	1.2	1.0	100[w]	EA
Imperial 10-6	1.1	1.5	96	E
Snowball Y	1.2	1.0	98	M
Suprimax RS	1.6	1.6	100	EA
Spring Snow	1.0	2.9	100[w]	M
White Dove	1.0	1.0	no curds	M
Snowball 99	1.7	1.2	56	EA
Snowball 123	1.3	1.2	86	M
White Contessa	flowered shortly after planting			E
Early Snowball A	1.9	1.3	44[w]	M
Snowflower	1.6	1.1	20	M
White Top	1.3	2.1	20	M
Christmas White	1.2	2.2	80	I
Andes	1.2	1.0	20[w]	I

Table 2.2. continued

	Visual rating[y]			
Cultivar	week 2	week of curd evaluation	Curd evaluation (% marketable)	Class
Snowball E	1.8	1.1	72	EU
Atos	1.7	1.3	84	I
White Fox	1.2	1.4	70	I
Super Snowball	1.7	1.4	24	I
White Rock SG-112	1.2	3.3	87	I
Self-Blanche	1.6	1.4	66	I
Snowball 34	1.7	2.3	36	I
White Empress	1.4	1.1	98	I
Racket	1.1	1.8	98[w]	I
$LSD_{0.05}$	0.2[z]	0.4[z]	9.0[z]	

[z]Mean separation within columns by LSD, P=0.05. Data presented are the means of 50 observations.

[y]Symptoms: 1=no symptoms; 2=slight tipburn on margins; 3=tipburn, necrosis prevalent extending in from margins, curling may be present; 4=tipburn extends to midrib, necrosis, curling; 5=severe necrosis, leaf development reduced.

[w]Fifty percent or more of the curds of these cultivars were less than 15 cm in diameter.

[x]Class from solution culture experiment: E=efficient; EA=efficient at accumulation only; M=moderately efficient; EU=efficient at utilization only; I=inefficient.

CHAPTER 3

ROOT-CA AFFINITY, GROWTH HABITS, AND LOW-CA TISSUE REQUIREMENTS ARE IMPORTANT CHARACTERISTICS OF CA-EFFICIENT CAULIFLOWER; CULTIVARS VARY IN SENSITIVITY TO H+ AND AL

3.1 Introduction

Tipburn in cauliflower was originally reported as scorch of winter cauliflower by Jenkinson and Campbell (1957). After growers in Western Massachusetts reported similar symptoms in the fall of 1978, Maynard et al. (1981) identified tipburn as a Ca-deficiency disorder. Since then, attempts have been made to reduce or eliminate tipburn by applying Ca to the soil as gypsum (Rosen et al., 1987) and foliar sprays (Rosen, 1990). Neither gypsum nor foliar sprays significantly reduced the incidence of tipburn however. In other *Brassica* species, the occurrence of tipburn has been effectively reduced by utilizing genotypic variations in susceptability (Nieuwhof, 1960). Solution culture screening has shown that variations exist among cauliflower strains and cultivars for tolerance to low Ca (Hochmuth, 1984; Chapter 2). The severity of tipburn displayed by the efficient cultivars grown under low to moderate Ca concentrations in the field, however, was not

significantly different from inefficient cultivars. The percent of marketable curds for efficient cultivars was uniformally higher than for inefficient cultivars (Chapter 2).

A number of environmental factors have been reported to influence the occurrence of tipburn (Collier and Tibbetts, 1982). These factors focus on soil fertility or conditions that influence the plants' transpiration rate and root pressure. In addition, low-Ca concentrations are commonly seen under acidic conditions where toxic concentrations of Al, Mn, and H+ are said to be responsible for deficiency symptoms and reduced growth (Jackson, 1967; Foy et al., 1978; 1987; Adams, 1981; Fageria et al., 1988).

Cauliflowers' response to low-Ca solution culture in comparison to low-Ca field conditions indicates that other aspects of Ca efficiency need to be examined to ascertain its usefulness in helping to alleviate the incidence of tipburn in the field.

The objectives of these studies were to establish Ca concentrations under which significant differences in dry weights could be realized; determine the significance Ca accumulation, Ca transport, and growth habit play in the Ca efficiency trait; and evaluate selected cultivars for tolerance to Al and H+.

3.2 Materials & Methods

Hydroponic set-up. Seeds from selected commercial cultivars of *Brassica oleracea,* botrytis group were germinated in perlite soaked once with modified Hoagland (1950) nutrient solution (0.07 mM KH_2PO_4, 0.15 mM $MgSO_4$, 0.36mM $NaNO_3$, 3.57 uM KCl, 1.79 uM H_3BO_3, 0.36uM $MnSO_4 \cdot H_2O$, 0.15 uM $ZnSO_4$, 0.04 uM $CuSO_4$, 0.07 uM $(NH_4)_6MO_7O_{24} \cdot 4H_2O$, and 2.86uM Fe in the form of FeEDTA). Calcium (125 uM) was supplied from $CaCl_2$. Approximately 18-21 days after sowing, seedlings were transferred to individual 1.5 liter polyethylene vessels containing 1.4 liters of nutrient solution (5 mM KNO_3, 1mM KH_2PO_4, 2mM $MgSO_4$, 5mM $NaNO_3$, 50 uM KCl, 25uM H_3BO_3, 5 uM $MnSO_4$ H_2O, 2uM $ZnSO_4$, 5uM $CuSO_4$, 1.5 uM $(NH_4)_6Mo_7O_{24}$ $4H_2O$, and 40 uM Fe as FeEDDHA). Calcium was supplied by $CaCl_2$ and varied according to each experiment. Each seedling was suspended in nutrient solution by supporting the stem of the seedling with a foam plug placed in a hole in the lid of the polyethylene vessel. Filtered air was bubbled continuously through the nutrient solution in each vessel for the duration of each experiment. Each vessel was lined with a black sheet of polyethylene to deter algal growth by reducing light penetration. The original volume of solution was maintained by adding deionized water daily. Light intensity and daylength were supplemented with high-intensity sodium lamps to achieve an irradiance level, two meters from the light source, of 380 umol $s^{-1} \cdot m^{-2}$ for a

period of twelve hours each day.

Experiments were conducted in a glasshouse located at the University of Massachusetts, Amherst, Ma . Temperatures in the glasshouse were set at 24 C days and 16 C nights.

All plant material was dried at 65 C in a forced-air drying oven. Seeds were ground in a mortar and pestle and tissue was ground in a Wiley mill to pass a 20-mesh screen. Samples and seeds were wet-ashed with HNO_3 and H_2O_2. Ca analysis was carried out by atomic absorption spectrophotometry (Greweling, 1976).

Seed analysis

Five replications of 10 seeds from cultivars 'Improved Holland Erfurt', 'Imperial 10-6', 'Self-Blanche', and 'Snowball 34' were washed with deionized water, ground, weighed and analyzed for Ca.

Seedling analysis

At the time the seedlings were normally transferred to individual polyethylene vessels, seedlings were removed from the perlite, and cotyledons and any residual seed coat were removed. Five replications of 10 seedlings were analyzed for Ca for each cultivar.

Dry weight accumulation under 13 Ca treatments

Five replications of the four cultivars previously mentioned were germinated in perlite and grown in hydroponic solution culture as outlined ealier. Seedlings were arranged in a randomized complete block design. Seventy-five, 150, 225, 300, 375, 450, 525, 675, 825, 975, 1225, 3375, or 7125 umol Ca was added to the nutrient solution in each polyethylene vessel. Plants were allowed to grow for approximately 5 weeks before being removed from vessels. Dry weight was recorded for each plant.

Periodic sampling with two Ca levels

Cultivars 'Self-Blanche', 'Snowball 34', 'Improved Holland Erfurt', and 'Imperial 10-6' were grown hydroponically in a randomized complete block design. Three hundred and seventy-five umol or 7000 umol of Ca was added to the nutrient solution prior to transferring the seedlings. Beginning after two weeks in solution culture, 10 plants of each cultivar were removed on a weekly basis for five weeks. Ca analysis was done for all plants. For the first 2 samplings, whole individual plants were analyzed because of insufficient growth for accurate readings of individual roots and shoots. Roots and shoots were analyzed separately for the remaining 3 samplings.

Calcium uptake under 4 pH regimes

The 4 cultivars mentioned previously were germinated in perlite and grown hydroponically in a split block design. One Ca treatment of 375 umol was maintained at 4 pH regimes of 4.5, 6.5, 8.5, or no control. Twenty plants of each cultivar were divided equally among the 4 pH regimes. The pH of the solution was adjusted twice daily with HCl or NaOH to maintain the original pH. pH readings were recorded daily from the noncontrolled vessels. Root and shoot dry weights were determined after five weeks followed by Ca analysis for individual plant parts. Total dry matter, total Ca, and CaER (Ca efficiency ratio) were calculated for each plant.

Aluminum study

Cultivars 'Improved Holland Erfurt, 'Imperial 10-6', 'Self-Blanche', 'Snowball 34', and 'Snowball E' were germinated in perlite and allowed to grow for 25 days. The additional 4 to 7 days in the perlite allowed for greater uniformity in root length within cultivars. The perlite was soaked twice with the nutrient solution described ealier because of the extended time in the perlite: when seeds were sown and again 12 days later. The Ca concentration of this solution was increased to 825 umol Ca to insure root growth would not be hindered prior to transferring seedlings to individual vessels. Nutrient solution containing 1250 umol

Ca was added to each vessel and 207 umol Al from $Al_2(SO_4)_3$ $18H_2O$ was added to one half the polyethylene vessels. Solution pH was adjusted twice daily to 4.5 with 0.1 N KOH or HCl in all vessels containing Al.

The nutrient solution in the vessels without Al was not adjusted. A total of 10 seedlings of each cultivar were grown in solution culture for 10 days. Plants were then remove from solution and root lengths recorded. The appearance of dark root tips was also noted.

3.3 Results

Seed analyis showed cultivar 'Imperial 10-6' to have significantly higher Ca content in the seed than 'Improved Holland Erfurt', 'Self-Blanche' and 'Snowball 34'.

Seedlings of 'Imperial 10-6' also contained a higher Ca content than the other three cultivars (Table 3.1).

Results-Dry weight accumulation under 13 Ca treatments

Significant differences in dry matter accumulation between Ca-efficient cultivars and Ca-inefficient cultivars were initially seen at Ca levels between 12 mg Ca and 135 mg Ca (Figure 3.1). At 135 mg Ca 'Self-Blanche' was similar to the efficient cultivars, and all were similar at 285 mg Ca. No differences in dry matter accumulation were seen between

cultivars 'Imperial 10-6' and 'Improved Holland Erfurt' at any of the 13 Ca treatments (Figure 3.1).

Results-Periodic sampling with 2 Ca levels

In the periodic sampling experiment, highly significant differences were observed at the low-Ca treatment for total dry matter (Figure 3.2) and total Ca accumulated between Ca-efficient cultivars 'Improved Holland Erfurt' and 'Imperial 10-6', and Ca-inefficient cultivars 'Self-Blanche' and Snowball 34' (Table 3.2). Differences were observed at week 5 and 6 for total dry matter produced. Differences in total accumulated Ca were seen at weeks 4, 5 and 6. 'Improved Holland Erfurt' also had significantly higher Ca at week 5 than 'Imperial 10-6 (Table 3.2). No differences were observed for total dry matter produced between cultivars at the full-Ca treatment (Figure 3.2). Total Ca accumulation was significantly lower for 'Snowball 34' at week 4 (Table 3.3). This was the only exception to a similar trend in Ca accumulation seen for all cultivars in full-Ca solution (Figure 3.3).

Growth in low-Ca solution yielded significantly different CaERs among Ca-efficient and Ca-inefficient cultivars (Figure 3.4). Differences between Ca-efficient cultivars were observed at weeks 3 and 6 in low-Ca solution (Figure 3.4). The largest increase in CaERs for Ca-inefficient cultivars were observed from weeks 5 to 6 at

low-Ca (Figure 3.4) and full-Ca (Figure 3.5). There was a ten-fold increase in CaER between low-Ca and full-Ca treatments.

In low-Ca solution, variations in dry matter produced per week shows efficient cultivars produced significantly more dry matter at weeks 4 and 5 than Ca-inefficient cultivars (Figure 3.6). Ca accumulated by each cultivar per week in low-Ca solution varied significantly (Figure 3.7). When grown in full-Ca solution, 'Improved Holland Erfurt' and 'Imperial 10-6', showed very similar patterns for dry matter production per week (Figure 3.8) and Ca accumulation per week (Figure 3.9). Similar patterns of dry matter production per week by Ca-inefficient cultivars were also observed (Figure 3.8). The largest weekly dry matter production for all cultivars was at week 6. Calcium efficient cultivars however, produced significantly greater dry matter at week 4 than Ca-inefficient cultivars (Figure 3.8). Calcium accumulated per week by Ca-inefficient plants in full-Ca solution varied for week 4 but were similar for 2,3,5 and 6 (Figure 3.9). In full-Ca solution, Ca accumulated per week was highest for all cultivars at or before the first sampling, week 2. Both Ca-inefficient cultivars showed a significant loss in Ca at week 6 (Figure 3.9).

Analysis of individual roots and for weeks 4,5, and 6 show different rates of Ca accumulation exists between low-

Ca and high-Ca treatments. In low-Ca solution, Ca content of the root did not vary significantly between samplings or cultivars. Ca content of efficient cultivars, however, was slightly greater than Ca-inefficient cultivars and uniformity can be seen among the two groups for the three weeks (Figure 3.10). In full-Ca solution, roots of all cultivars accumulated large concentrations of Ca initially. This concentration declined with each sampling (Figure 3.10). No differences between cultivars within each teatment and sampling were observed for this same period. Shoot Ca content of plants grown in low-Ca increased through the sampling period and were greatest for efficient cultivars (Figure 3.11). In full-Ca solution, shoot Ca contents were drastically higher than shoots from low-Ca solution and levels increased over the three sampling periods (Figure 3.11).

Differential increases in shoot Ca between weeks 4 and 5 were not offset by loss of Ca from the root in all cultivars in full-Ca solution. Between weeks 5 and 6, loss of Ca by the roots of Ca-inefficient cultivars can not be accounted for by the increase in shoot Ca content (Table 3.4). In low-Ca solution, Ca content accumulated per week by roots and shoots increased in all cultivars. Calcium efficient cultivars showed a 3 fold increase in Ca accumulated by the roots between weeks 4-5 and 5-6 (Table 3.5). Inefficient cultivars showed a 10 fold increase for

the same period. Calcium accumulated in the shoots for weeks 4-5 show 'Improved Holland Erfurt' accumulated significantly more Ca in the shoot than the other 3 cultivars (Table 3.5). All cultivars continued to produce root and shoot dry matter between weeks 4-5 and 5-6 in low and full-Ca solution (Table 3.6). Root growth for all cultivars was greatest in full-Ca solution between weeks 5-6. Increases in root growth can also be seen for this same period in low-Ca solution although this is not significant (Table 3.6). Calcium efficient cultivars showed a great deal of uniformity in shoot growth for each period in low-Ca solution. 'Imperial 10-6' was more uniformed in full-Ca solution. Calcium inefficient cultivars varied tremendously in shoot dry matter production between weeks and treatments (Table 3.6).

Results-Calcium uptake under 4 pH regimes

Cultivars varied in their ablity to change the solution pH in uncontrolled pots. Differences between efficient and inefficient cultivars were initially seen after 1 week. Later shoots developed on 3 of 5 replications of 'Self-Blanche' after 16 days. Solution pH for these plants continued to rise. The solution pH of the 2 remaining plants was unchanged for the duration of the experiment. All 4 cultivars varied in their ability to tolerate H^+. Total dry matter produced by 'Self-Blanche' and 'Imperial

10-6' showed significant reductions at pH 8.5. 'Snowball 34' was not significantly affected by any of the pH treatments although slight reductions can be seen for pH treatments 4.5 and 8.5 (Table 3.7). Differences in total dry matter produced by the 4 cultivars can be attributed to reduced shoot growth. Significant differences in shoot dry matter were realized at pH treatments corresponding to treatments where a reduction in total dry matter was also observed (Table 3.8). No significant differences were seen between treatments, however, for total root dry matter produced (Table 3.9). Total Ca accumulated by 'Improved Holland Erfurt' and 'Imperial 10-6' was reduced at pH 8.5. 'Snowball 34' showed a reduction at 4.5 and 8.5. 'Self-Blanche' was significantly affected by all 3 pH treatments (Table 3.10). Later shoot growth by 'Self-Blanche' occurred under all pH treatments but was more prevalent in pH 8.5. CaER for 'Improved Holland Erfurt' were significantly higher at pH 4.5 and 8.5. 'Self-Blanche' and 'Snowball 34' were slightly more efficient at pH 4.5. Imperial 10-6 showed no significant differences in CaER between treatments (Table 3.11). Highly significant linear affects were seen for total Ca, total dry matter produced, shoot dry matter, and CaER. Significant quadratic affects were noted for total Ca, root dry weight, and CaER (Tables 3.7-3.10).

Results-Aluminum study

Variations in tolerance to aluminum in solution culture were seen among 5 cultivars. All cultivars showed dark colored root tips. 'Improved Holland Erfurt', 'Snowball 34', and 'Snowball E' had significantly less root growth. 'Imperial 10-6 and 'Self-Blanche' were unaffected by the aluminum treatment (Table 3.12).

3.4 Discussion

Both Ca-efficient cultivars 'Imperial 10-6' and 'Improved Holland Erfurt' have a higher affinity for Ca when grown in low-Ca solution culture than Ca-inefficient cultivars 'Self-Blanche' and 'Snowball 34'. Growth habit also appears to be an important factor in Ca-efficiency in cauliflower seedlings. Both Ca-efficient cultivars initiated shoot growth earlier in low-Ca and full-Ca solutions than Ca-inefficient cultivars and may indicate a much lower Ca requirement for Ca-efficient cultivars. Greater initial shoot growth would allow Ca-efficient plants to have a higher rate of transpiration and photosynthesis leading to increased uptake and transport of other nutrient as well as Ca. In low-Ca solution culture, different mechanisms for efficiency appear to exist between Ca-efficient cultivars 'Imperial 10-6' and 'Improved Holland Erfurt'. Differences in CaER for these two cultivars between weeks 3 and 4 suggests 'Imperial 10-6' initially

utilizes Ca more efficiently. This could indicate a lower Ca-tissue requirement in the shoot of this cultivar. Also, the overall rate of growth and Ca accumulation shown by 'Imperial 10-6' in low-Ca was much less erratic than that displayed by 'Improved Holland Erfurt'. Initially, 'Improved Holland Erfurt' has a slightly higher rate of Ca accumulation than 'Imperial 10-6', and a higher rate of Ca transport to the shoot between weeks 4 and 5. A greater ability by this cultivar to transport Ca to the shoot may result from a higher rate of transpiration or a greater rate of exchange for other cations. Both would favor the upward translocation of Ca. Greater Ca accumulation coupled with slower growth due to a higher Ca requirement could explain the low CaER observed early-on for 'Improved Holland Erfurt' in relation to the other Ca-efficient cultivar, 'Imperial 10-6'. Different rates of Ca accumulation can also be distinguished, at full-Ca, between Ca-inefficient cultivars 'Self-Blanche' and 'Snowball 34'. 'Self-Blanche' accumulated significantly more Ca between weeks 3 and 4 than 'Snowball 34' yet both show similar rates of dry matter production.

The ability of the roots to continue to remove Ca from solution between weeks 4 and 5, in full-Ca solution, shows membrane integrity is adequate for root functioning. Between weeks 5 and 6 however, Ca transport from the roots to the shoots Ca-inefficient cultivars results in an

insufficient Ca concentration inside the roots to maintain membrane integrity. Both Ca-inefficient cultivars appear to require a greater internal Ca concentration in the roots than Ca-efficient cultivars in order to function properly.

Ca-efficient cultivars 'Improved Holland Erfurt' and 'Imperial 10-6' appear to be tolerant of high concentrations of H^+ in solution. Dry matter accumulation and Ca accumulation by these 2 cultivars were not affected by low pH. The reduced growth of Ca-inefficient cultivars in solution pH 4.5 may be attributed to H^+ interaction with Ca uptake and the inability of these cultivars to maintain the structural integrity of root membranes due to a higher Ca requirement. Other physiological processes related to shoot growth of inefficient cultivars may also have a higher degree of pH sensitivity than Ca-efficient cultivars. Ca uptake and Ca utilization by efficient cultivars was not affected by reduced shoot growth. At higher pH, efficient cultivars were able to utilization Ca more efficiently to offset reduced Ca uptake. This allowed them to maintain dry matter production. Reduced growth by all cultivars at pH 8.5 can be attributed to $CaPO_4$ precipitation. A lower Ca content would be expected for all cultivars. Ca-efficient cultivars however, have a higher affinity for Ca and would be able to remove more free-Ca from solution. Ca content for these cultivars would be higher than Ca-inefficient plants but lower than their respective controls.

Ca-inefficient cultivars were unable to remove significant amounts of Ca from the initial low-Ca solution. Ca availability to these plants was reduced further by $CaPO_4$ precipitation. Na toxicity may also be a factor that contributed to reduced growth. 'Improved Holland Erfurts' inability to tolerate Al in solution culture shows Ca-efficiency and tolerance to H^+ does not guarantee Al tolerance. Although root growth of Ca-inefficient 'Self-Blanche' was unaffected by Al, other physiological aspects relating to uptake, transport, and shoot growth appear to be pH sensitive. Ca accumulation for this cultivar was significantly reduced by all pH treatments but no reduction in root dry matter was observed. The loss of apical dominance by 'Self-Blanche' suggests a Ca/auxin interaction may be important in the translocation of Ca to the shoot tip in this cultivar.

Calcium efficiency in cauliflower appears to be a root mediated function coupled with growth habits and lower Ca tissue requirements that accentuate Ca efficiency. Growth habits differ between Ca-efficient cultivars and can be influenced by external factors.

Table 3.1 Ca content of individual seeds and seedlings of 4 cauliflower cultivars[x].

CULTIVAR	SEED Ca CONTENT mg/seed	SEEDLING Ca CONTENT mg/seeding
Improved Holland Erfurt	0.12	0.05
Imperial 10-6	0.22	0.09
Self-Blanche	0.14	0.04
Snowball 34	0.13	0.04
$LSD_{0.05}$[x] =	0.04	0.03

[x]Mean separation within columns by LSD, P=0.05. Data presented are the means of 5 observations.

Table 3.2 Total Ca content of 4 cauliflower cultivars sampled weekly for 5 weeks after 2 initial weeks in low-Ca nutrient solution culture.

Cultivar	Week/quartiary root total Ca content (mg)				
	2	3	4	5	6
Improved Holland Erfurt	1.32	1.48	1.60	1.84	1.87
Snowball 34	1.19	1.20	1.44	1.38	1.53
Imperial 10-6	1.25	1.42	1.60	1.68	1.83
Self-Blanche	1.16	1.34	1.31	1.37	1.54
$LSD._{0.05x}$	0.073				

[x]Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Table 3.3 Total Ca content of 4 cauliflower cultivars sampled weekly for 5 weeks after 2 initial weeks in full-Ca nutrient solution culture.

Cultivar	Week/quartiary root total Ca content (mg)				
	2	3	4	5	6
Improved Holland Erfurt	3.49	3.49	3.81	3.79	3.82
Snowball 34	3.50	3.56	3.53	3.85	3.75
Imperial 10-6	3.43	3.48	3.76	3.88	3.72
Self-Blanche	3.48	3.48	3.76	3.88	3.72

$LSD_{0.05x}$= 0.073

[x]Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Table 3.4 Weekly change in root and shoot Ca content of 4 cauliflower cultivars grown in full-Ca nutrient solution culture.

	Change in Ca content (mg)			
	Weeks 4-5		Weeks 5-6	
Cultivar	Shoot	Root	Shoot	Root
Improved Holland Erfurt	+ 32.38	- 36.80	+ 60.00	- 51.39
Snowball 34	+ 40.56	+ 20.77	+ 48.47	- 69.35
Imperial 10-6	+ 37.08	- 22.95	+ 47.96	- 48.74
Self-Blanche	+ 60.28	- 32.63	+ 29.73	- 65.28

$LSD_{0.05}$=30.91

Mean separation by LSD, P=0.05. Data presented is the mean of 5 observations.

Table 3.5 Weekly change in root and shoot Ca content of 4 cauliflower cultivars grown in low-Ca nutrient solution culture.

	Change in Ca content (mg)			
	Shoots		Roots	
cultivar	week 4-5	week 5-6	week 4-5	week 5-6
Improved Holland Erfurt	4.77	0.74	.05	.16
Snowball 34	0.00	1.84	.01	.06
Imperial 10-6	1.32	3.01	.03	.10
Self-Blanche	0.63	2.04	.00	.05
$LSD_{0.05}$=	2.11 shoots		0.36 roots	

Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Table 3.6 Weekly change in root and shoot dry matter produced by 4 cauliflower cultivars grown in low-Ca or full-Ca nutrient solution culture.

	Change in dry matter ln (g dry wt + 1)							
	low Ca				full Ca			
	week 4-5		week 5-6		week 4-5		week 5-6	
cultivar	shoot	root	shoot	root	shoot	root	shoot	root
Improved Holland E.	1.35	.05	1.61	.07	1.19	.00	2.26	.56
Snowball 34	.76	.04	1.12	.13	2.03	.16	1.45	.36
Imperial 10-6	.34	.05	1.35	.19	1.77	.01	1.95	.52
Self-Blnch.	.40	.00	1.60	.23	1.77	.15	2.00	.49

$LSD_{0.05}$=.35

Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Table 3.7 Total dry matter produced by 4 cauliflower cultivars grown in low Ca nutrient solution culture with 4 pH treatments[x].

pH treatment	Cultivar/ Total dry matter (g) Improved Holland Erfurt	Self Blanche	Imperial 10-6	Snowball 34
No Control	4.51	1.30	4.52	0.94
4.5	5.10	0.85	4.67	0.44
6.5	4.64	0.80	4.28	0.77
8.5	4.49	0.49	3.02	0.42
	$LSD_{0.05}$[x]=0.59			

[x]Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Regression analysis Total dry weight

Q	D	SS	MSE=0.322 DF=12	MS	F
-13.0	40	4.225	linear	4.225	13.1211**
+0.6	120	0.003	quadratic	0.003	0.0093[ns]

Table 3.8 Shoot dry matter produced by 4 cauliflower cultivars grown in low-Ca nutrient solution culture with 4 pH treatments[x].

pH	Cutivar/Shoot dry matter (g)			
Treatment	Improved Holland	Self	Imperial	Snowball
	Erfurt	Blanche	10-6	34
No Control	4.04	1.08	4.00	0.74
4.5	4.63	0.69	4.19	0.33
6.5	3.39	0.63	3.72	0.61
8.5	4.01	0.37	2.60	0.28

$LSD_{0.05}$[x]=0.53

[x]Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Regression analysis shoot dry weight

Q	D	SS	MSE=0.262	DF=12	MS	F
-12.8	40	4.10		linear	4.10	15.634**
+2.0	120	0.0333		quadratic	0.0333	0.127ns

Table 3.9 Root dry matter produced by 4 cauliflower cultivars grown in low-Ca nutrient solution culture with 4 pH treatments[x].

	Cultivar/Root dry matter (mg)			
pH	Improved Holland	Self	Imperial	Snowball
TREATMENT	Erfurt	Blanche	10-6	34
No Control	0.47	0.22	0.52	0.20
4.5	0.47	0.17	0.49	0.11
6.5	0.45	0.21	0.59	0.16
8.5	0.47	0.12	0.42	0.14

$LSD_{0.05}$[x]=0.20

[x]Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Regression analysis root dry weight

Q	D	SS	MSE=.006	DF=12	MS	F
-0.4	40	0.004		linear	.004	.667
-2.0	120	0.033		quadratic	.033	5.550*

Table 3.10 Total Ca accumulated by 4 cauliflower cultivars grown in low-Ca nutrient solution culture with 4 pH treatments[x].

	Cultivar/Total Ca accumulated (mg)			
pH	Improved Holland	Self	Imperial	Snowball
TREATMENT	Erfurt	Blanche	10-6	34
No Control	9.87	6.12	10.16	4.25
4.5	9.55	2.92	9.71	1.44
6.5	8.75	3.61	9.21	3.23
8.5	7.90	1.74	6.62	1.62

$LSD_{0.05}$[x]=1.16

[x]Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Regression analysis total Ca

Q	D	SS	MSE=0.322	DF=12	MS	F
-13.0	40	4.225		linear	4.225	13.121**
+0.6	120	0.003		quadratic	0.003	0.009ns

Table 3.11 CaER (Ca efficiency ratios) of 4 cauliflower cultivars grown in low-Ca nutrient solution culture with 4 pH treatments[x].

pH	Cultivar/CaER			
	Improved Holland	Self	Imperial	Snowball
Treatment	Erfurt	Blanche	10-6	34
No Control	457	221	446	229
4.5	535	294	484	308
6.5	424	225	475	238
8.5	572	221	446	229
	$LSD_{0.05}$[x]=66			

[x]Mean separation by LSD, P=0.05. Data presented are the means of 5 observations.

Regression analysis CaER

Q	D	SS	MSE=4893.538	DF=12	MS	F
6559	40	1075512.025		linear	1075512.025	219.782**
2339	120	45591.008		quadratic	45591.008	9.317*

Table 3.12. Root length of 5 califlower cultivars grown for 10 days in nutrient solution culture containing 207 umol Al and 1250 umol Ca.

	TREATMENT/Root length (mm)	
	Aluminum	No
Cultivar	207umol	aluminum
Improved Holland Erfurt	6.60	9.26
Snowball 34	5.50	8.60
Imperial 10-6	6.61	7.50
Self-Blanche	5.14	5.50
Snowball E	5.00	13.20

$LSD_{0.05}$[x]=2.12

[x]Mean separation by LSD, P=0.05. Data presented is the mean of 5 observations.

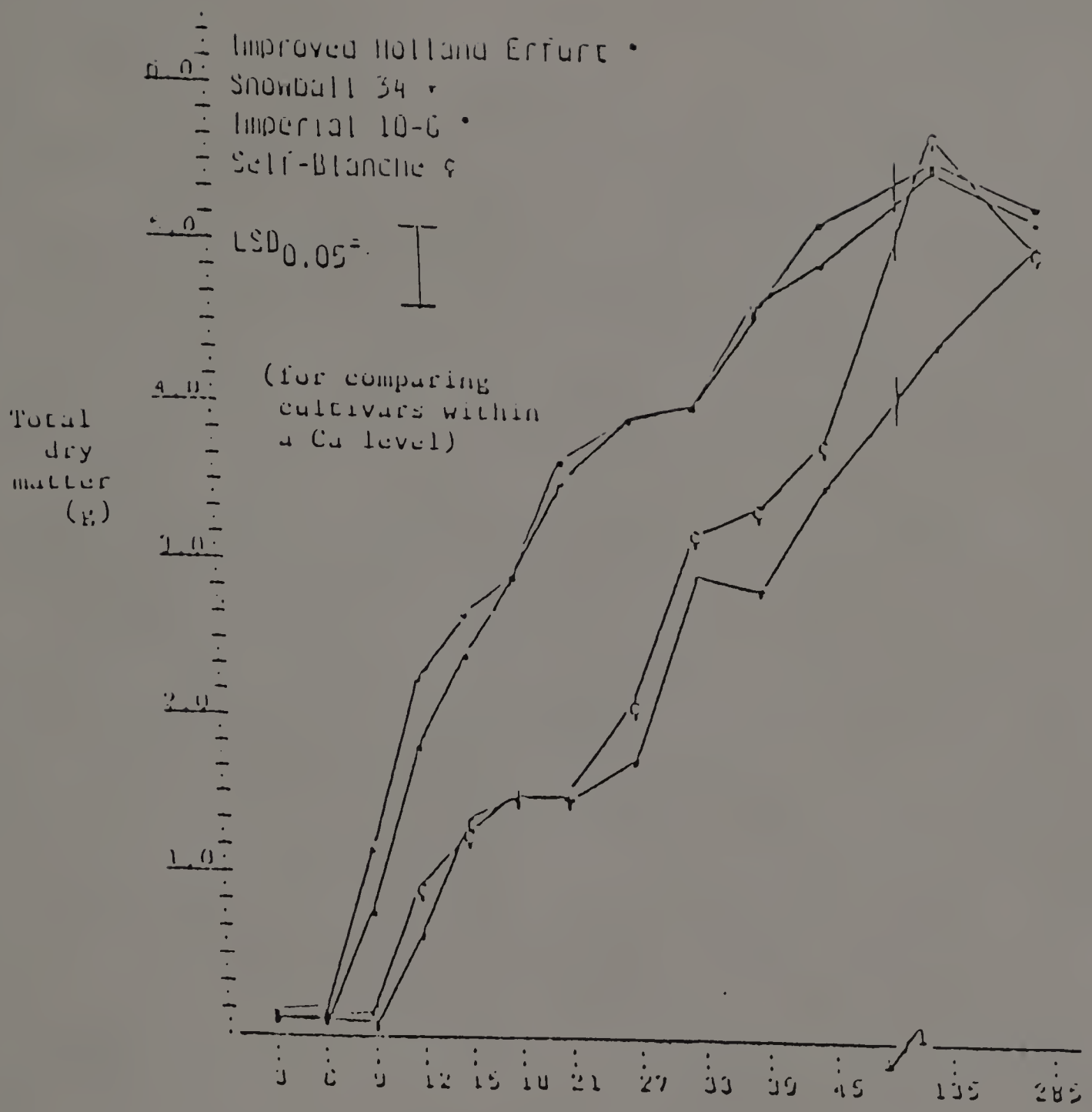

Figure 3.1 Dry matter produced by 4 cauliflower cultivars grown in solution culture containing 13 Ca treatments.

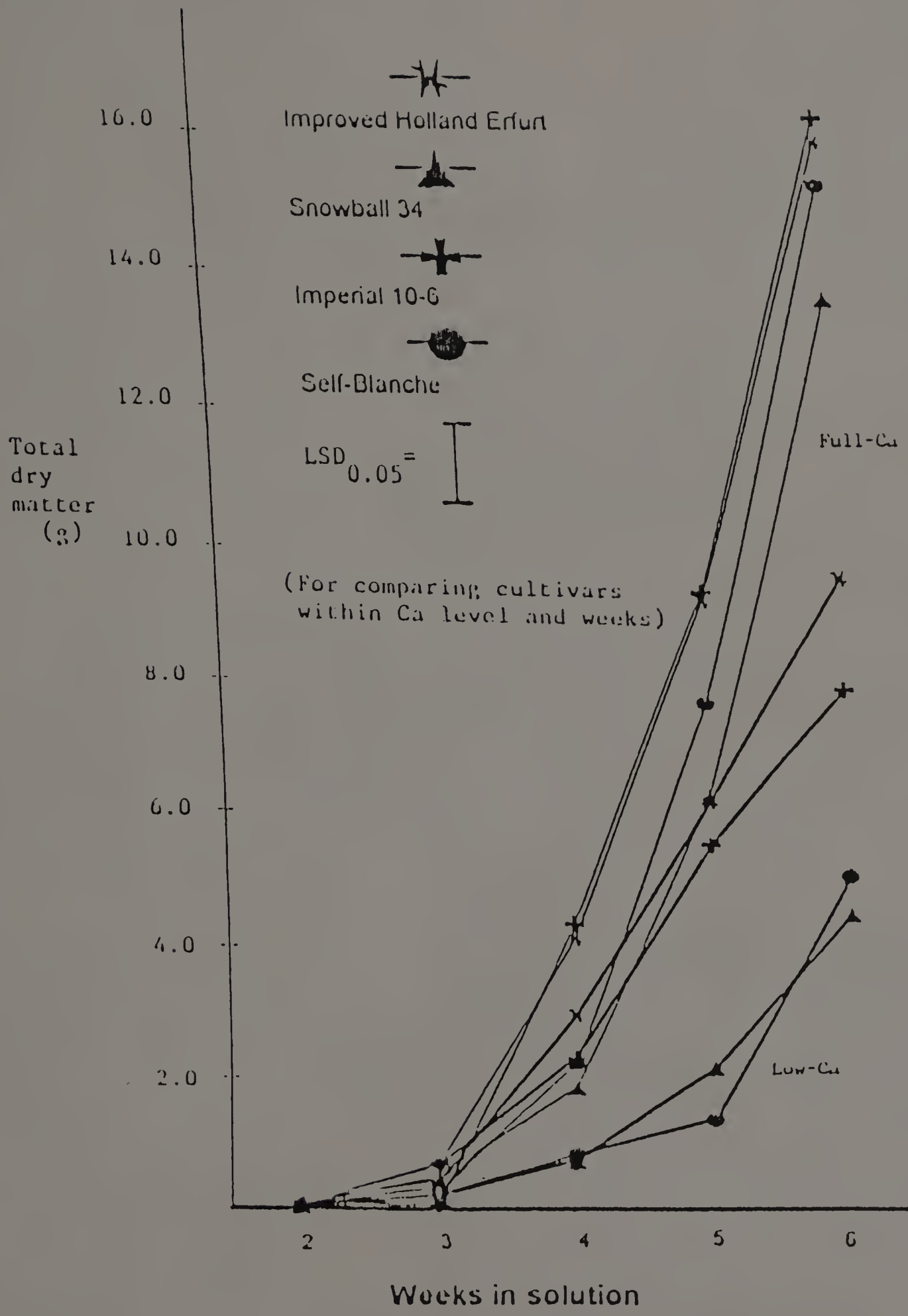

Figure 3.2 Dry matter produced by 4 cauliflower culitars grown in low-Ca or full-Ca solution for 6 weeks starting at week 2.

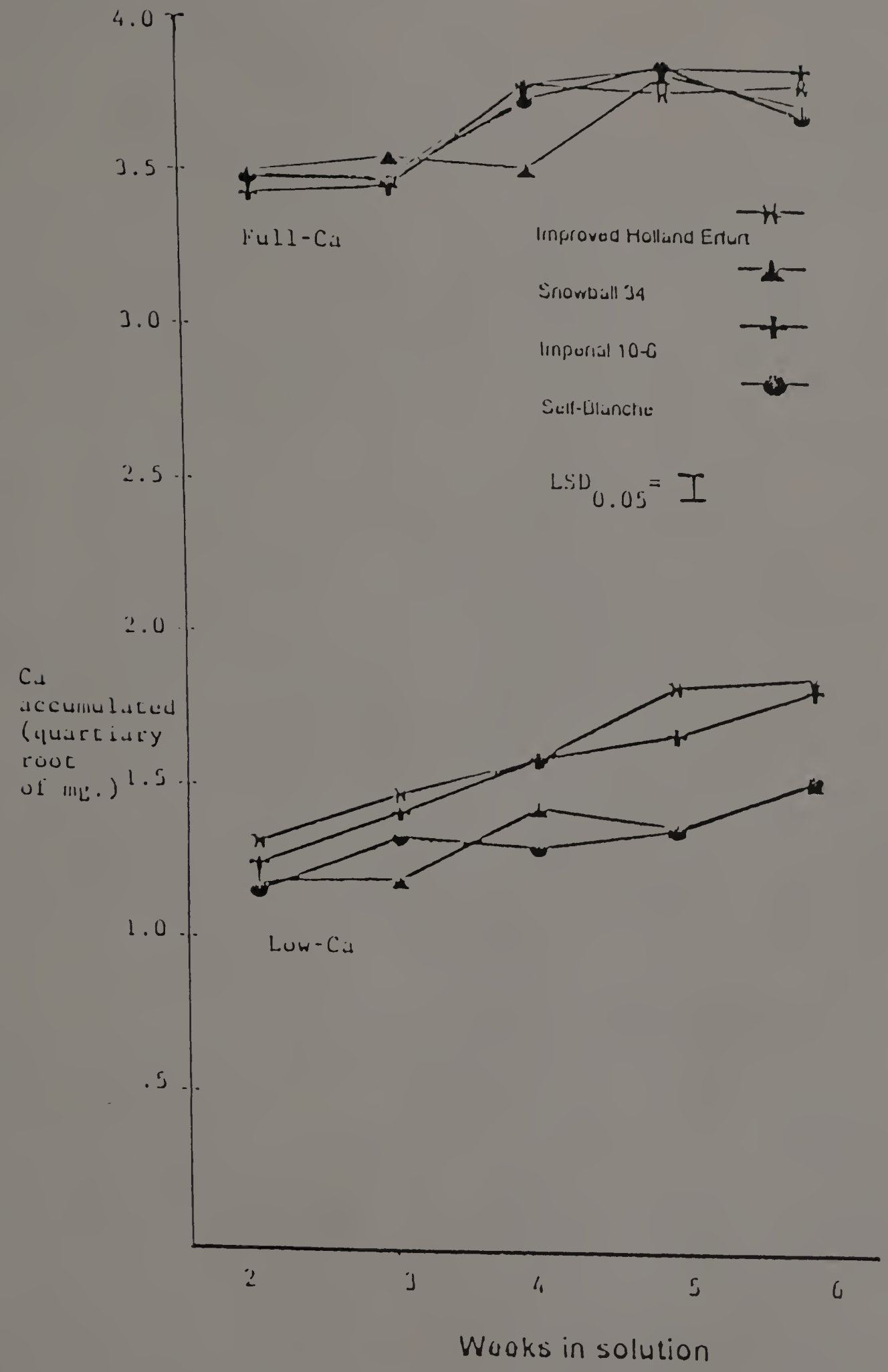

Figure 3.3 Ca accumulated by 4 cauliflower cultivars grown in low-Ca or full-Ca nutrient solution sampled weekly for 5 weeks after 2 initial weeks.
Expressed as the quartiary root of mg. Ca.

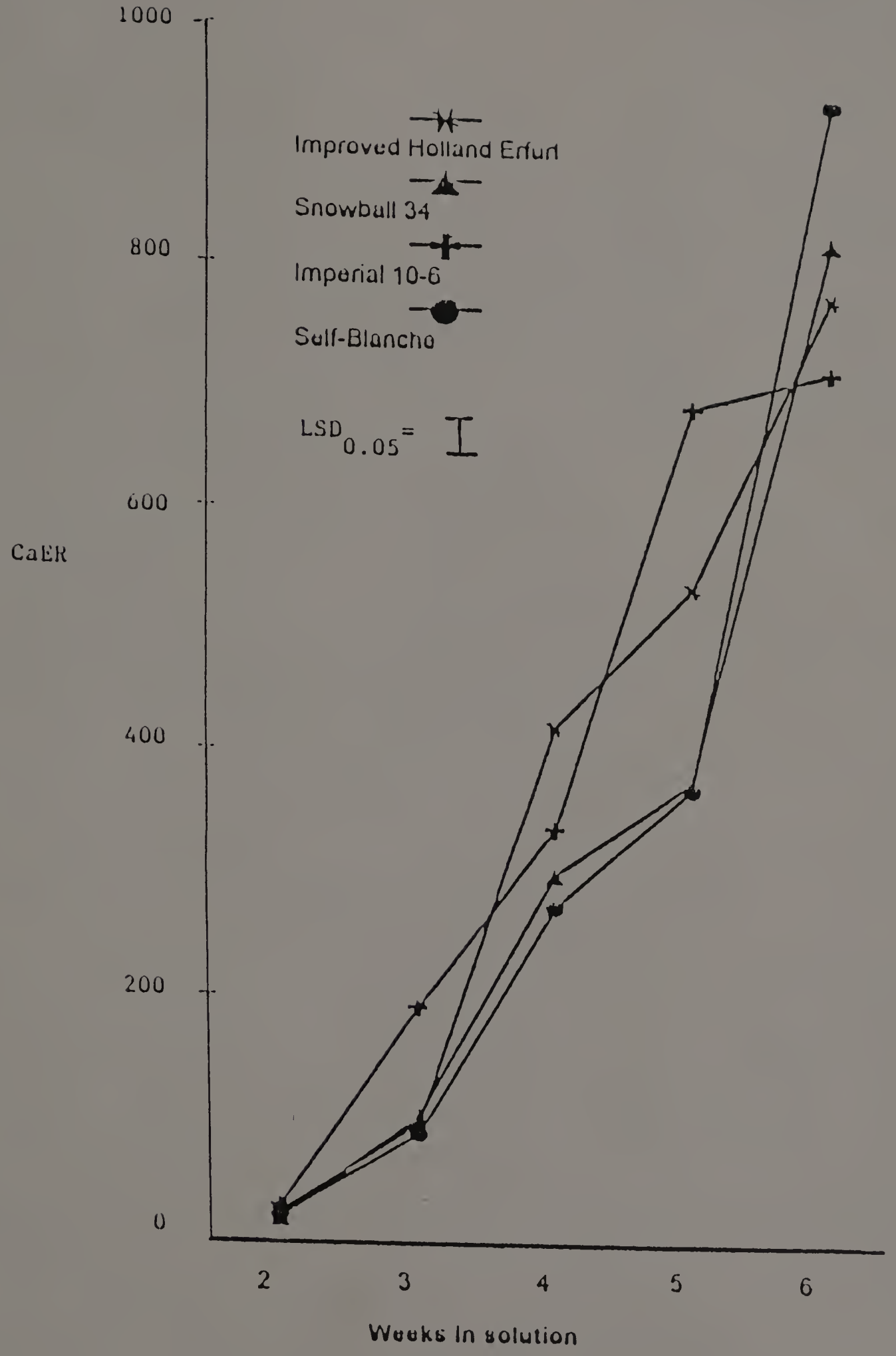

Figure 3.4 CaER of 4 cauliflower cultivars grown in low-Ca nutrient solution sampled weekly for 5 weeks after 2 weeks.

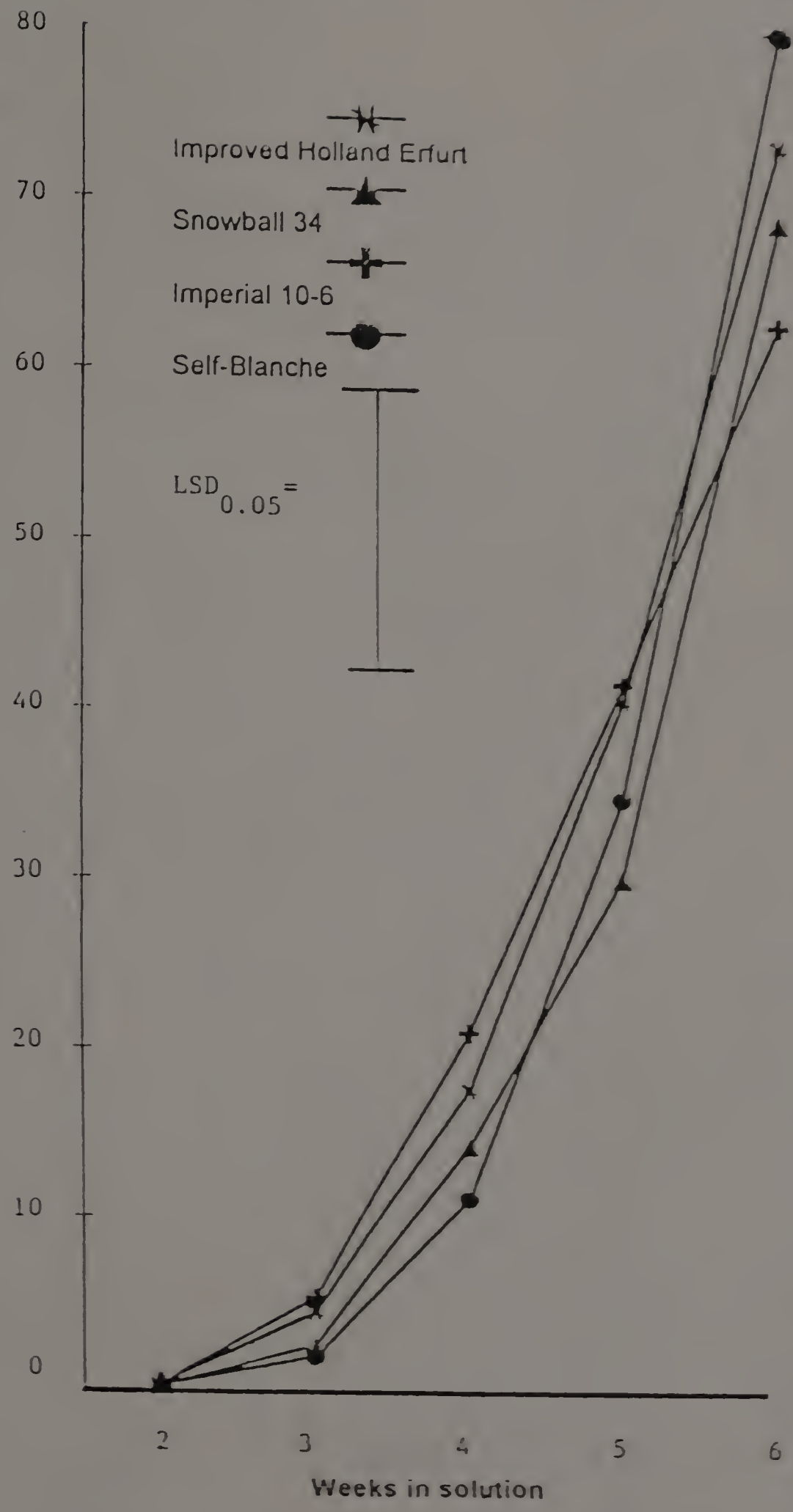

Figure 3.5 CaER of 4 cauliflower cultivars grown in full-Ca nutrient solution sampled weekly for 5 weeks after 2 initial weeks.

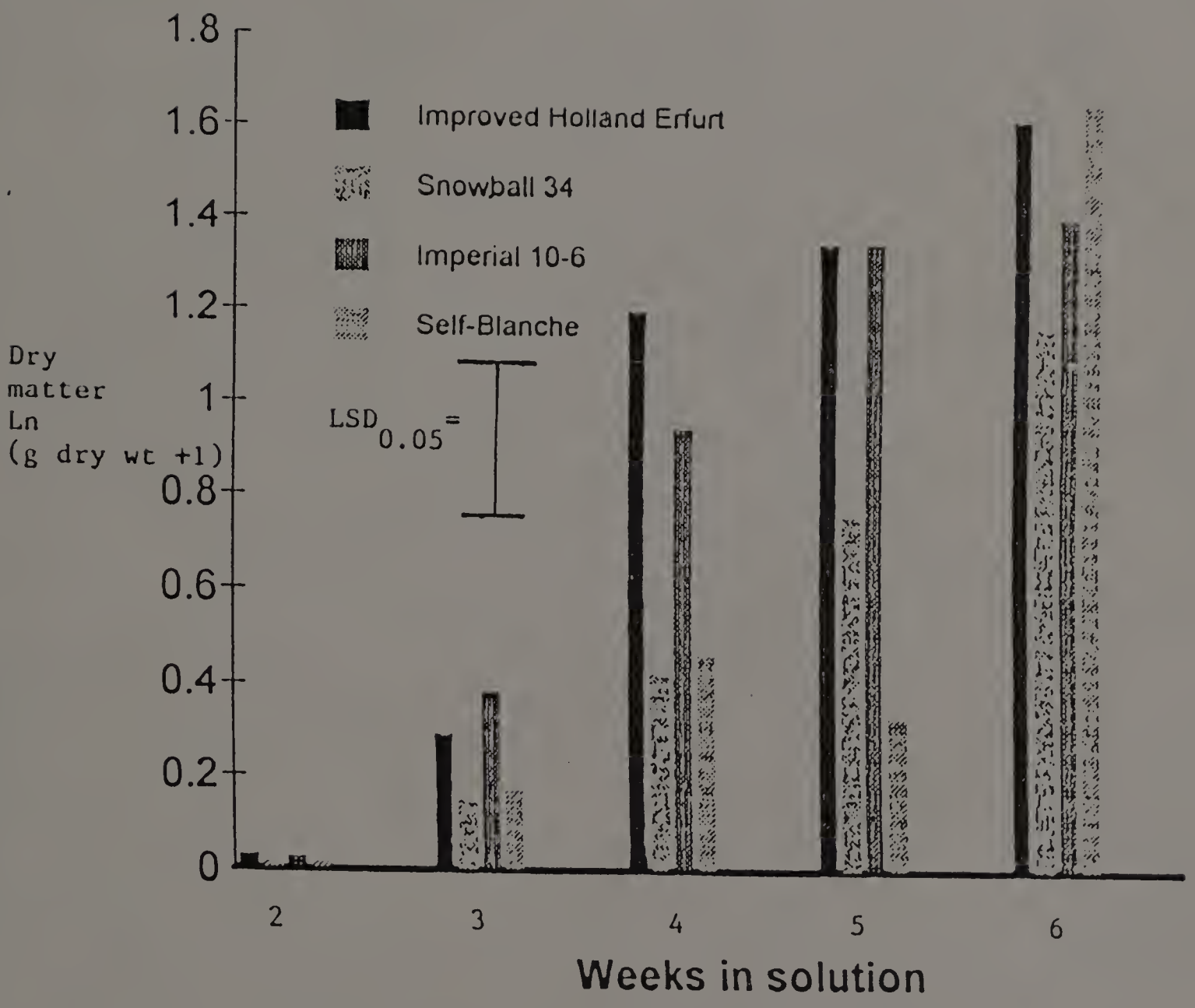

Figure 3.6 Dry matter produced per week by 4 cauliflower cultivars grown in low-Ca nutrient solution sampled weekly for 5 weeks after 2 initial weeks. Expressed as Ln (gram dry wteight +1)

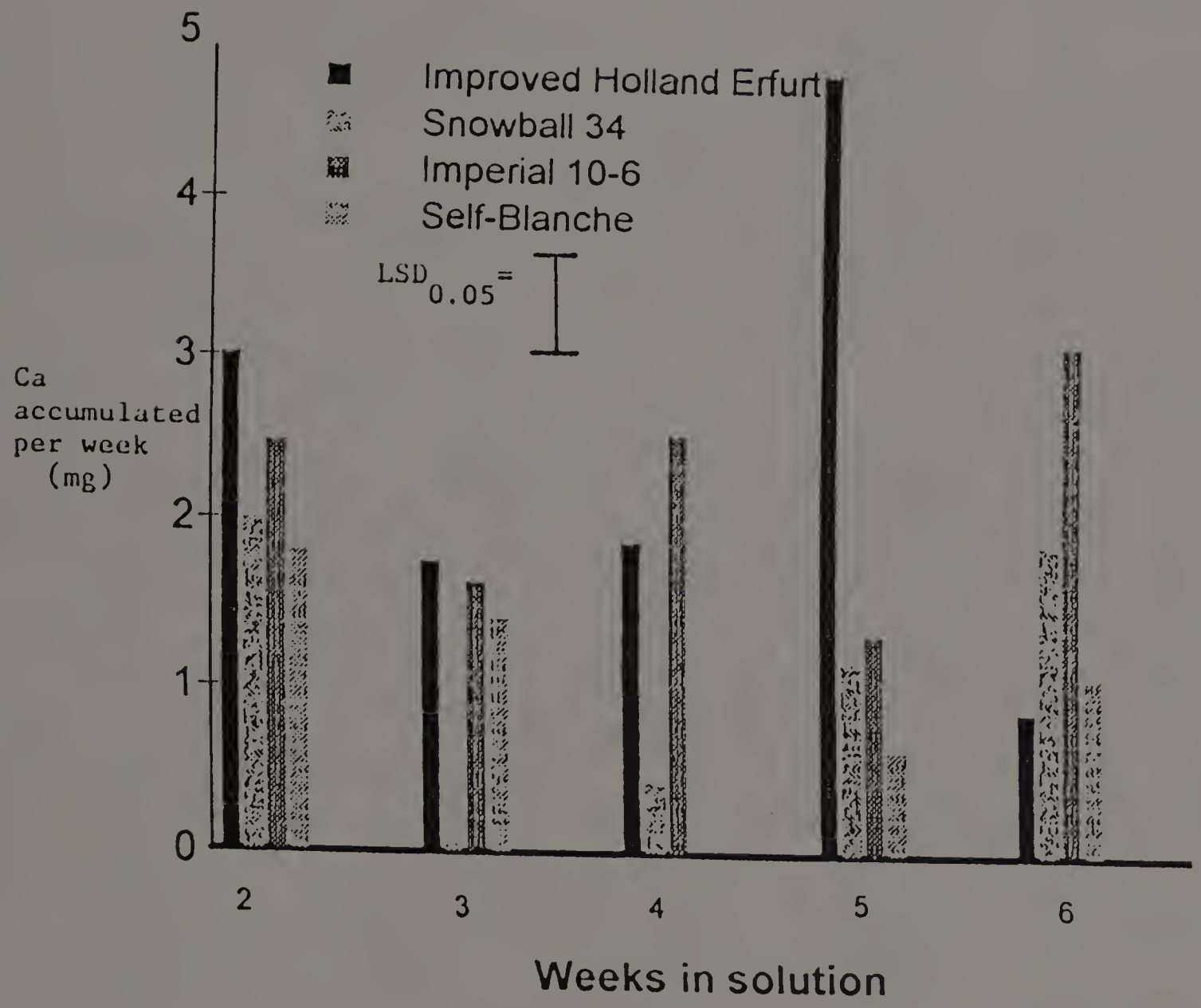

Figure 3.7 Ca accumulated per week by 4 cauliflower cultivars grown in low-Ca nutrient solution sampled weekly for 5 weeks after 2 initial weeks.

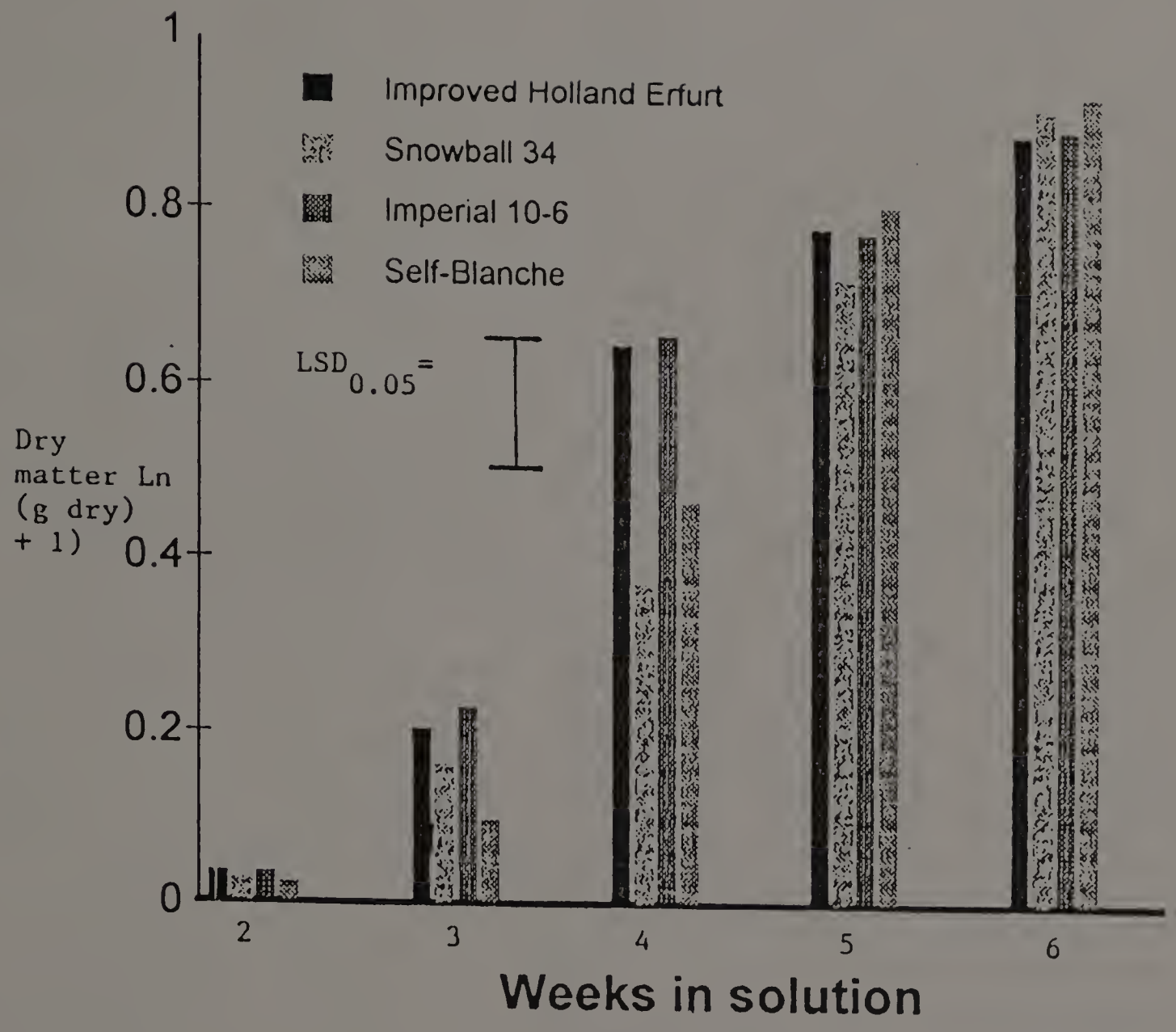

Figure 3.8 Dry matter produced per week by 4 cauliflower cultivars grown in full-Ca nutrient solution sampled weekly for 5 weeks after 2 initial weeks. Expresses as Ln (gram dry weight +1)

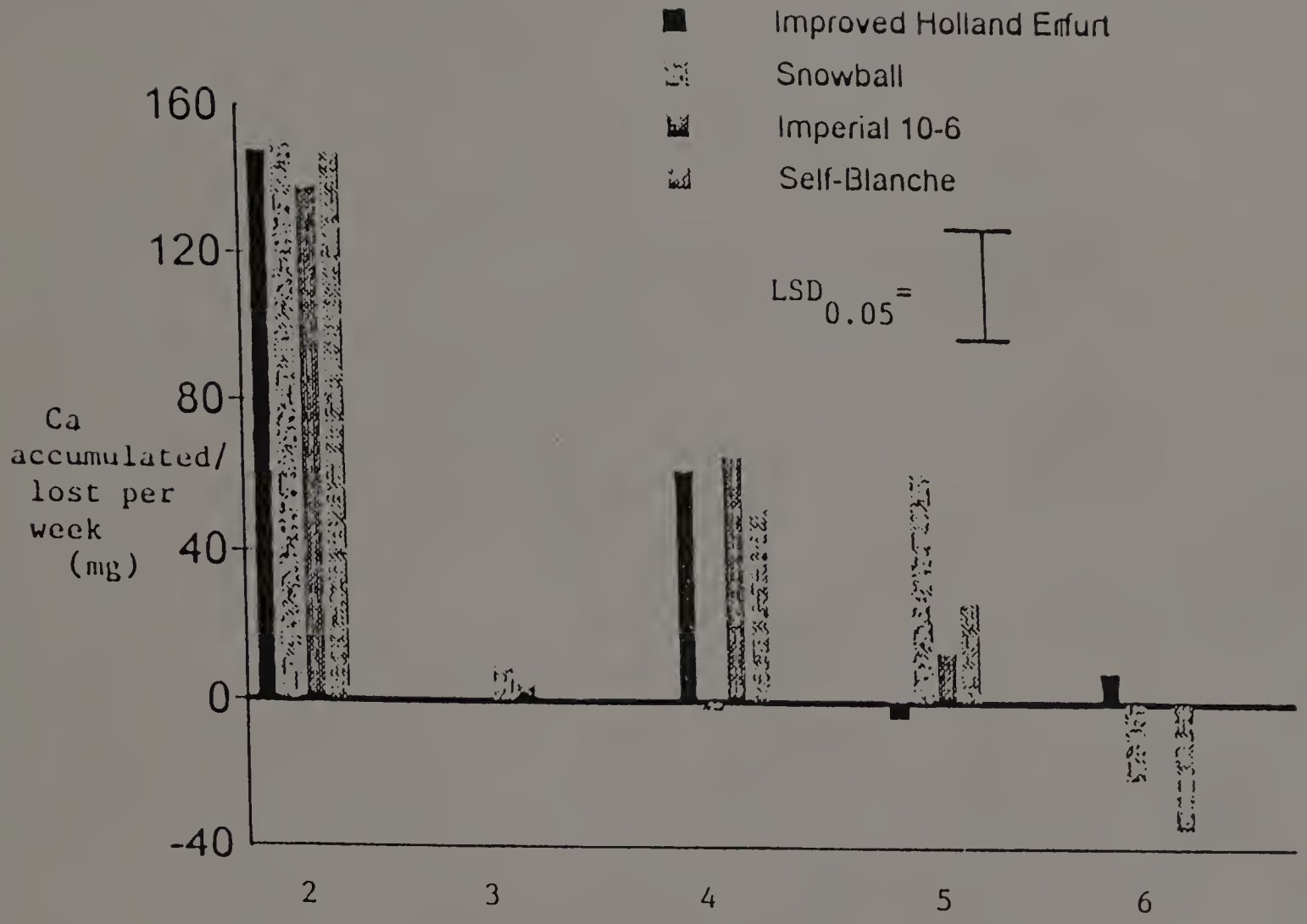

Figure 3.9 Change in Ca content per week by 4 cauliflower cultivars grown in full-Ca nutrient solution sampled weekly for 5 weeks after 2 initial weeks.

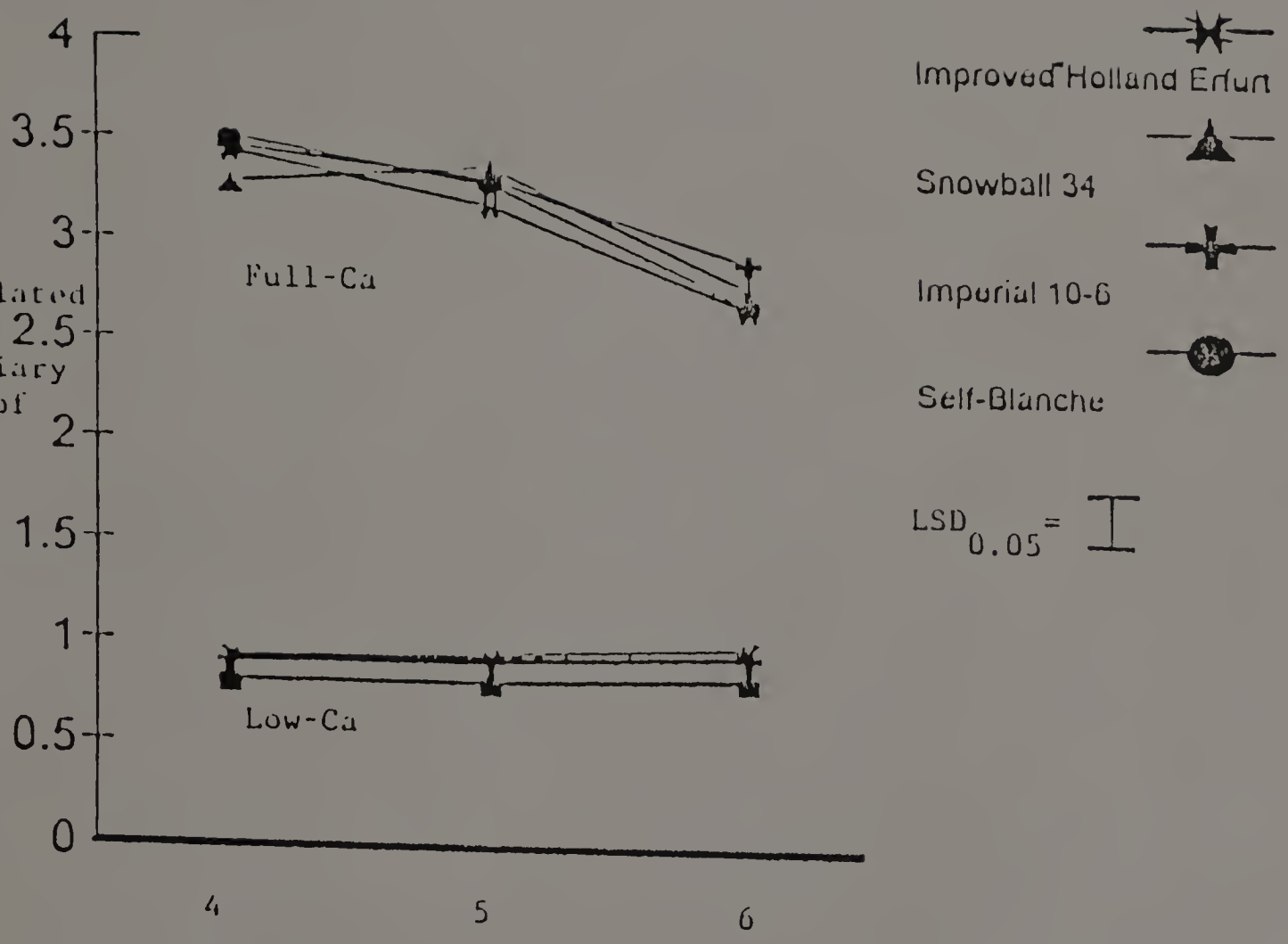

Figure 3.10 Ca accumulated by roots of 4 cauliflower cultivars grown in low-Ca or full-Ca nutrient solution sampled weekly for 3 weeks after 4 initial weeks.
Expressed as the quartiary root of mg. Ca.

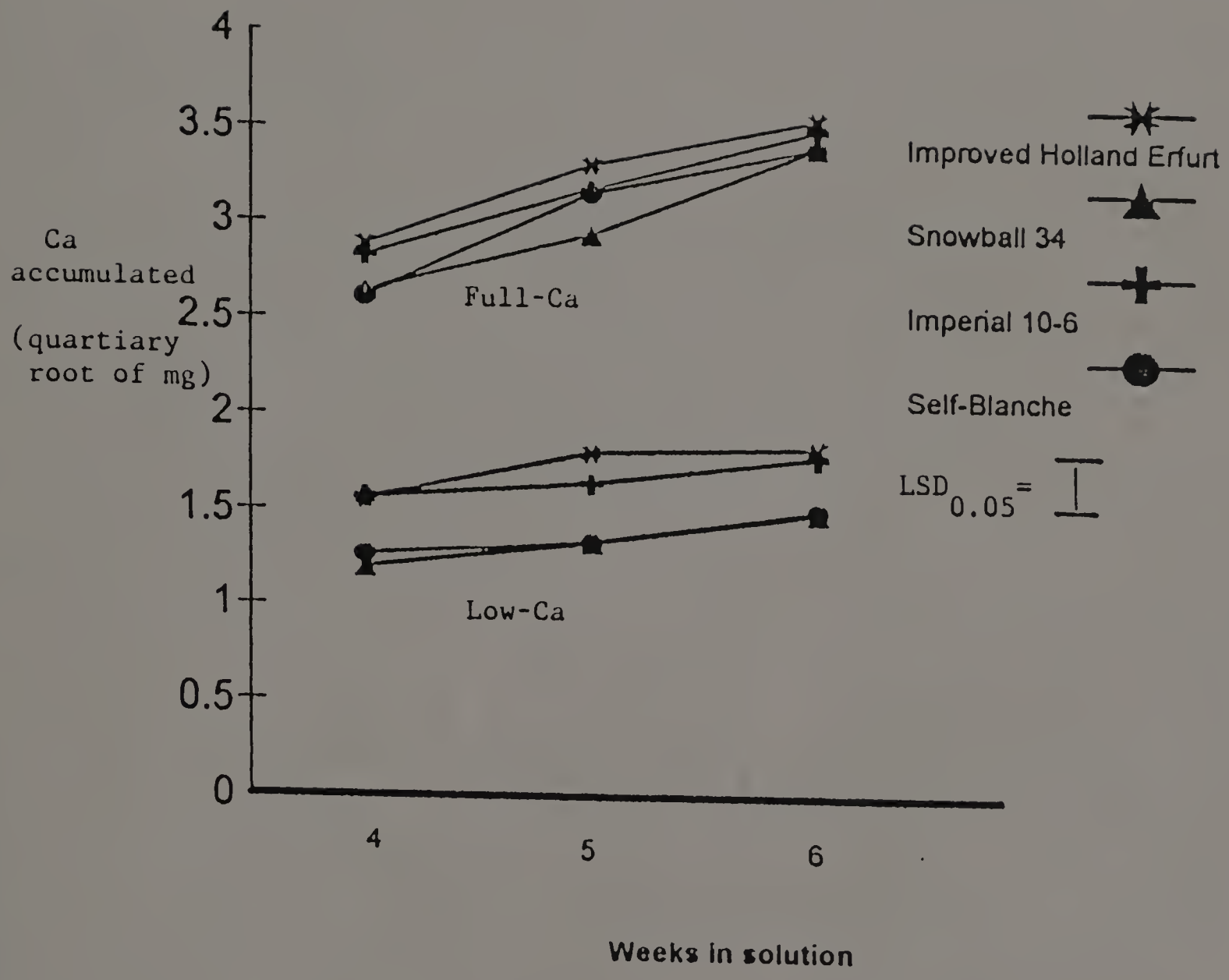

Figure 3.11 Ca accumulated by shoots of 4 cauliflower cultivars grown in low-Ca or full-Ca nutrient solution sampled weekly for 3 weeks after 4 initial weeks.
Expressed as the quartiary root of mg. Ca.

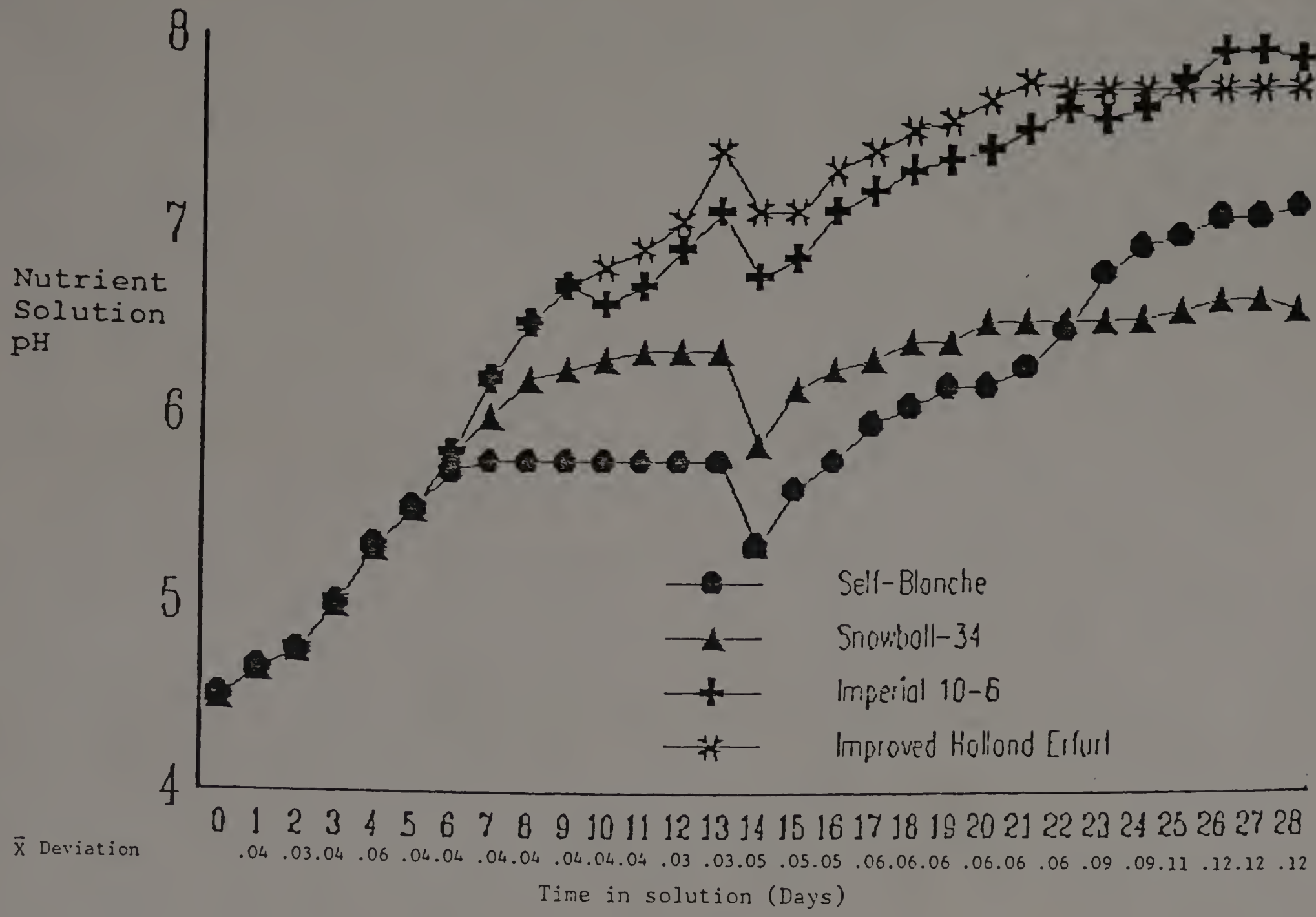

Figure 3.12 Daily pH measurements of nutrient solution in which 4 cauliflower cultivars were grown for 28 days.

CHAPTER 4

F1 POPULATIONS OF CAULIFLOWER WERE UNIFORMLY CA-EFFICIENT

4.1 Introduction

Several review articles describing genetic differences in plant nutrition were published in the early 1960s (Myers, 1960; Vose, 1963; Gerloff, 1963; Epstein and Jefferies, 1964). More recently, Vose (1981) published an extensive review dealing with the effects of genotypic factors on plant nutrient requirements. Differences in nutrient accumulation have been shown to be genetically controlled. Ca concentrations in the earleaf of corn have been reported to be under the control of three genes acting in an additive manner (Gorsline et al., 1964; 1968). Naismith et al. (1974) suggested that the genetic loci influencing Ca, phosphorus, and magnesium accumulation in corn is present on chromosome nine. The resistance to blossom end rot, a Ca disorder of tomatoes, has been found to be a recessive trait (Greenleaf and Adams, 1969). Resistance could be based on lower requirements for Ca in the fruit, or greater efficiency with which the plant accumulates Ca in the fruit. It was suggested that there is an association between the

genotype for uniform fruit ripening and the high incidence of blossom-end rot (Trinklein and Lambeth, 1976). Longeragan and Snowball (1969) compared the Ca concentrations of eighteen different species of plants grown in solutions with those grown in the field and found little difference in the Ca content of a particular species, whether the plant was grown in soil or solution culture. Myers (1960) reviewed the literature on genotypic variations within and between species for element content and concentration, and concluded that cultivars showed marked variations in the accumulation of different elements, indicating the presents of independent mechanisms under different genetic control. Vose (1963) showed that differential nutrient uptake and differential tissue requirements for elements are primary forms of nutrient efficiency. The identification of these sites of ion uptake and the understanding of the mechanisms involved with this process are important for a better understanding of the physiological basis of genotypic differences in nutrient requirements.

4.2 Materials and Methods

Seeds of 'Improved Holland Erfurt', 'Self-Blanche', 'Improved Holland Erfurt' x 'Self-Blanche' and 'Self-Blanche' x 'Improved Holland Erfurt' were germinated in perlite soaked once with modified Hoagland and Arnon (1950)

nutrient solution (0.07 mM KH_2PO_4, 0.15 mM $MgSO_4$, 0.36 mM $NaNO_3$, 3.57 uM KCl, 1.79 uM H_3BO_3, 0.36 uM $MnSO_4$ H_2O, 0.15 uM $ZnSO_4$, 0.04 uM $CuSO_4$, 0.07 uM $(NH_4)_6Mo_7O_{24}$, and 2.86 uM Fe in the form of FeEDTA). Calcium (125 uM) was supplied from $CaCl_2$.

Approximately 18-21 days after sowing, single seedlings were transferred to individual 1.5-liter polyethylene vessels containing 1.4 liters of nutrient solution (5 mM KNO_3, 1 mM KH_2PO_4, 2 mM $MgSO_4$, 5 mM $NaNO_3$, 50 uM KCl, 25 uM H_3BO_3, 5 uM $MnSO_4$ H_2O, 2 uM $ZnSO_4$, 5 uM $CuSO_4$, 1.5 uM $(NH_4)_6Mo_7O_{24}$ $4H_2O$, and 40 uM Fe as FeEDTA). Calcium (375 uM) was suppied from $CaCl_2$. Each vessel was lined with a black polyethylene sheet to deter algal growth by reducing light penetration. Each seedling was suspended in nutrient solution by supporting the stem of the seedling with a foam plug placed in a hole in the lid of the polyethylene vessel. Filtered air was bubbled continuously through the nutrient solution in each vessel for the duration of the experiment. The original volume of solution was maintained by adding deionized water daily. Light intensity and daylength were supplemented with high-intensity sodium lamps to achieve an irradiance level, two meters from the light source, of 380 umol s^{-1} m^{-2} for a period of twelve hours each day. Fifteen plants of each parent and each cross were arranged in a randomized complete block experiment and allowed to grow for five weeks in a glasshouse located at the University of

Massachusetts, Amherst, MA during the months of April and May in 1984. Temperatures in the glasshouse were set at 24C days and 16C nights. Plants were visually rated for Ca deficiency symptoms, removed from the vessels. All plant material was dried at 65C in a forced-air drying oven. Analysis of variance was conducted for dry matter accumulated.

4.3 Results and Discussion

No significant differences were seen between dry weights accumulated by the F1 populations. Dry weights were significantly different between F1 plants and parental plants. Significant differences also existed between the 2 parents (Table 4.1).

The uniformity between the 2 F1 populations suggests the Ca efficiency trait is dominant and nuclear in nature. The increase in dry weight between the F1 hybrids and the Ca-efficient parent, 'Improved Holland Erfurt', may be attributed to heterosis.

Table 4.1 Dry weight accumulated by 2 F1 populations of cauliflower seedlings and the parents, grown for 5 weeks in nutrient solution containing 375 umol Ca^{x}.

Parent cultivars		F1 E x I	F1 I x E
Improved Holland Erfurt	Self Blanche	Improved Holland Erfurt x Self-Blanche	Self-Blanche x Improved Holland Erfurt
4.56	1.54	5.71	5.75
$LSD_{0.05}^{x} = 0.16$			

[x]Mean separation between groups by LSD, P =0.05. Data presented are the means of 15 observations.

APPENDIX

ANALYSIS OF VARIANCE DATA

A.1 ANOVA-Table 2.1
26 cauliflower cultivars grown in low-Ca solution culture

Visual symptoms

Source	DF	SS	MS	F
Total	103	248.2885		
R	3	52.5145	17.5048	
T	25	146.2885	5.8515	8.8686**
RT	75	49.4855	0.6598	

Total dry matter

Source	DF	SS	MS	F
Total	103	439.3937		
R	3	13.7864	4.5955	
T	25	387.5643	15.5026	30.5651**
RT	75	38.0430	0.5072	

Total plant calcium

Source	DF	SS	MS	F
Total	103	698.8409		
R	3	0.8492	0.2831	
T	25	624.7170	24.9887	25.577**
RT	75	73.2747	0.9770	

A.1 CONTINUED ANOVA-Table 2.1

Ca efficiency ratios

Source	DF	SS	MS	F
Total	103	2,366,589.92		
R	3	8,365,159.99	2,786,053.33	
T	25	2,321,811.30	92,872.45	191.251**
RT	75	36,420.46	485.61	

A.2 ANOVA-Table 2.2
26 cauliflower cultivars grown under low-Ca field conditions

Visual leaf symptoms week 2

Source	DF	SS	MS	F
Total	119	24.0737		
R	4	12.0737	3.0184	
T	23	7.8837	0.3428	9.7386**
RT	92	3.2409	0.0352	

Visual leaf symptoms at curd evaluation

Source	DF	SS	MS	F
Total	119	58.712		
R	4	1.848	0.4620	
T	23	47.176	2.0511	19.4789**
RT	92	9.688	0.1053	

Curd evaluation % marketablility

Source	DF	SS	MS	F
Total	119	67,350.5917		
R	4	1,847.7167	461.9292	
T	23	60,262.1917	2,620.0953	45.9957**
RT	92	5,240.6833	56.9639	

A.3 ANOVA-Table 3.1

A.3.1 Seed Ca analysis of 4 cauliflower cultivars

Ca content

Source	DF	SS	MS	F
Total	19	1.0086		
Treatment	3	0.5363	0.1788	223.50**
Replication	4	0.4631	0.1158	
T x R	12	0.0092	0.0008	

A.3.2 Ca seedling analysis of 4 cauliflower cultivars

Source	DF	SS	MS	F
Total	19	0.0149		
Treatment	3	0.0085	0.0028	5.60**
Replication	4	0.0004	0.0001	
R x V	12	0.0060	0.0005	

A.4 ANOVA-Figure 3.1

4 cauliflower cultivars grown in solution culture under 13 different Ca concentrations

Total plant dry matter

Source	DF	SS	MS	F
Total	259	951.0325	3.6719	
Treatment	12	756.2429	63.0202	113.2032**
Replication	4	11.4274	2.8569	5.1318*
Variety	3	76.3548	25.4516	96.9954**
T x R	48	26.7208	0.5567	
T x V	36	41.5166	1.1532	4.3948*
R x V	12	0.9891	0.0824	0.3140^{ns}
T x V x R	144	37.7809	0.2624	

A.5 ANOVA-Table 3.2, Table 3.3, Figure 3.3
Total Ca content of 4 cauliflower cultivars sampled weekly for 5 weeks after 2 initial weeks in low-Ca or full-Ca solution

Total quartiary root of mg Ca accumulated

Source	DF	SS	MS	F
V	3	0.9918846	0.3306282	40.05**
T	1	241.8355287	241.8355287	72680.35**
V x T	3	0.6045546	0.2015182	34.49**
H	4	4.6811514	1.1702878	153.65**
V x H	12	0.2807575	0.0233965	3.40**
T x H	4	0.2404698	0.0601175	10.57**
V x T x H	12	0.4439609	0.0369967	10.75**
R	4	0.0980530	0.0245132	
V x R	12	0.0990604	0.0082550	
T x R	4	0.0133095	0.0033274	
V x T x R	12	0.0701050	0.0058421	
H x R	16	0.1219650	0.0076166	
V x H x R	48	0.3307153	0.0068899	
T x H x R	16	0.0909660	0.0056854	
VxTxHxR	48	0.1651383	0.0034404	

A.6 ANOVA-Table 3.4
Weekly change in root and shoot Ca content of 4 cauliflower cultivars grown in full-Ca nutrient solution

Change in Ca content

Source	DF	SS	MS	F
V	3	1583.1704	527.7235	3.34*
H	1	6779.2984	6779.2984	8.71*
V x H	3	7154.8645	2384.9548	4.74*
P	1	137284.1070	137284.1070	329.91**
V x P	3	1980.3948	660.1316	1.30ns
H x P	1	10009.2328	10009.2328	12.37*
V x H x P	3	5912.5166	1970.8389	2.01ns
R	4	401.0513	100.2628	
V x R	12	1894.9608	157.9134	
H x R	4	3111.9553	777.9888	
V x H x R	12	6036.4506	503.0376	
R x P	4	1695.3558	423.8389	
V x R x P	12	6089.0729	507.4227	
H x R x P	4	3237.5901	809.3975	
V x H x R x P	12	11785.9259	982.1605	

A.7 ANOVA-Figure 3.2
Dry matter produced by 4 cauliflower cultivars sampled weekly for 5 weeks after 2 initial weeks in low-Ca or full-Ca nutrient solution

Total plant dry matter

Source	DF	SS	MS	F
Total	199	4775.1077		
treatment	1	416.1324	416.1324	1308.5925**
Variety	3	95.9319	31.9773	21.4858**
Harvest	4	3339.2695	834.8174	263.2746**
Replication	4	44.9407	11.2352	
T x V	4	36.7634	9.1908	20.0235**
T x H	4	512.2455	128.061	84.6404**
T x R	4	1.2731	0.318	
V x H	12	81.7993	6.8166	5.5469**
V x R	12	17.8592	1.4883	
H x R	16	50.7343	3.1709	
T x V x R	12	5.5089	0.459	
T x H x R	16	24.2028	1.513	
V x H x R	48	58.9891	1.2289	
T x V x H	12	57.7452	4.8121	7.28**
T x V x H x R	48	31.7124	0.661	

A.8 ANOVA-Figure 3.4, Figure 3.5
CaER of 4 cauliflower cultivars sampled weekly for 5 weeks after 2 initial weeks in low-Ca or full-Ca nutrient solution initial weeks

Ca efficiency ratios (CaER)

Source	DF	SS	MS	F
Total	199	16738465.83		
Treatment	1	8323988.842	8323988.842	2455.7879**
Variety	3	176189.091	58729.679	19.8979**
Harvest	4	6095613.624	1523903.406	262.8423**
Replication	4	84094.052	21023.513	
T x V	3	66384.756	22128.525	19.0444**
T x H	4	1084033.263	271008.316	56.8346**
V x H	12	175553.03	14629.419	2.8287**
T x R	4	13558.157	3389.539	
V x R	12	35418.594	2951.550	
H x R	16	92764.578	5797.786	
T x V x R	12	13943.142	1161.928	
T x H x R	16	76293.927	4768.37	
V x H x R	48	248248.187	5171.837	
T x V x H	12	131731.726	10977.644	4.3674**
T x V x H x R	48	120650.863	2513.56	

A.9 ANOVA-Table 3.5 - Shoots,
Weekly change in root and shoot Ca content of 4 cauliflower cultivars grown in low-Ca nutrient solution

Change in Ca content

Source	DF	SS	MS	F
V	3	31.3871675	10.46238917	11.10**
H	1	2.3571025	2.35710250	2.85ns
V x H	3	70.9648075	23.65493583	10.07**
R	4	1.1546600	0.28866500	
V x R	12	11.3097200	0.94247667	
H x R	4	3.3120600	0.82801500	
V x H x R	12	28.1852800	2.34877333	

A.10 ANOVA-Table 3.5 - Roots,
Weekly change in root and shoot Ca content of 4 cauliflower cultivars grown in low-Ca nutrient solution

Change in Ca content

Source	DF	SS	MS	F
V	3	0.0388475	0.01294917	17.78**
H	1	0.0497025	0.04970250	36.75**
V x H	3	0.0056075	0.00186917	2.76ns
R	4	0.0045600	0.00114000	
V x R	12	0.0087400	0.00072833	
H x R	4	0.0054100	0.00135250	
V x H x R	12	0.0081300	0.00067750	

A.11 ANOVA-Table 3.6
Weekly change in root and shoot dry matter produced by 4 cauliflower cultivars grown in low-Ca or full-Ca nutrient solution

Change in dry matter Ln (g dry wt + 1)

Source	DF	SS	MS	F
V	3	0.47223029	0.15741010	2.07 ns
T	1	6.42434517	6.42434517	277.84**
V x T	3	0.72437442	0.24145814	7.86**
H	1	3.85871902	3.85871902	141.52**
V x H	3	1.73603427	0.57867809	2.59 ns
T x H	1	0.00594039	0.00594039	0.02 ns
V x T x H	3	2.44689766	0.81563255	5.62*
P	1	69.32692704	69.32692704	5135.71**
V x P	3	0.54747126	0.18249042	6.89**
T x P	1	1.79771686	1.79771686	86.64**
V x T x P	3	0.56920311	0.18973437	8.85**
H x P	1	0.04271875	0.04271875	1.24 ns
V x H x P	3	0.92693933	0.30897978	4.51*
T x H x P	1	0.65564255	0.65564255	3.47 ns
V x T x H x P	3	0.71676009	0.23892003	4.09*
R	4	0.43832265	0.10958066	
V x R	12	0.91060354	0.07588363	
T x R	4	0.09249143	0.02312286	
V x T x R	12	0.36881770	0.03073481	
H x R	4	0.10906433	0.02726608	
V x H x R	12	2.68009182	0.22334098	
T x H x R	4	1.11269674	0.27817419	

A.11 continued ANOVA-Table 3.6 Weekly change in root and shoot dry matter produced by 4 cauliflower cultivars grown in low-Ca or full-Ca solution

Change in dry matter Ln (g dry wt + 1)

Source	DF	SS	MS	F
V x T x H x R	12	1.74212435	0.14517703	
R x P	4	0.05399597	0.01349899	
V x R x P	12	0.31794907	0.02649576	
T x R x P	4	0.08299386	0.02074847	
V x T x R x P	12	0.25712920	0.02142743	
H x R x P	4	0.13802390	0.03450598	
V x H x R x P	12	0.82219558	0.06851630	
T x H x R x P	4	0.75567185	0.18891796	
V x T x H x R x P	12	0.70031225	0.05835935	

A.12 ANOVA-Table 3.7
Total dry matter produced by 4 cauliflower cultivars grown in low-Ca nutrient solution with 4 pH treatments

Total dry weight

Source	DF	SS	MS	F
R	4	4.156	1.039	
T	3	6.575	2.192	6.802**
R x T	12	3.866	0.322	
V	3	253.921	84.640	158.639**
V x R	12	8.490	0.708	1.326**
T x V x R	48	25.610	0.534	
Total	79	302.619		

A.13 ANOVA-Figure 3.6, Figure 3.8
Dry matter produced per week by 4 cauliflower cultivars sampled weekly for 5 weeks after 2 initial weeks in low-Ca or full-Ca nutrient solution

Ln (gram dry wt + 1)

Source	DF	SS	MS	F
V	3	3.14820979	1.04940326	22.04**
T	1	9.04297591	9.04297591	440.15**
V x T	3	1.13971933	0.37990644	20.54**
H	3	42.44341585	14.14780528	356.64**
V x H	9	3.50560571	0.38951175	2.60*
T x H	3	1.84224578	0.61408193	3.94*
V x T x H	9	3.99226495	0.44358499	4.22**
R	4	1.04971254	0.26242813	
V x R	12	0.57135223	0.04761269	
T x R	4	0.08218021	0.02054505	
V x T x R	12	0.22191977	0.01849331	
H x R	12	0.47604170	0.03967014	
V x H x R	36	5.38771363	0.14965871	
T x H x R	12	1.87158951	0.15596579	
V x T x H x R	36	3.78124397	0.10503455	

A.14 ANOVA-Figure 3.7
Ca accumulated per week by 4 cauliflower cultivars sampled weekly for 5 weeks after 2 initial weeks in low-Ca nutrient solution

Total Ca accumulated

Source	DF	SS	MS	F
V	3	35.0560638	11.6853546	162.32**
H	3	6.8023938	2.2674646	2.16ns
V x H	9	104.7750712	11.6416746	7.09**
R	4	2.4653925	0.6163481	
V x R	12	0.8638675	0.0719890	
H x R	12	12.6249875	1.0520823	
V x H x R	36	59.0706725	1.6408520	

A.15 ANOVA-Figure 3.9
Change in Ca content per week by 4 cauliflower cultivars sampled weekly for 5 weeks after 2 initial weeks in full-Ca nutrient solution

Change in Ca content

Source	DF	SS	MS	F
V	3	1355.63007	451.87669	11.66**
H	3	36722.62109	12240.87370	15.11**
V x H	9	31638.69085	3515.41009	5.32**
R	4	696.07286	174.01821	
V x R	12	465.19183	38.76599	
H x R	12	9719.19431	809.93286	
V x H x R	36	23774.80652	660.41129	

A.16 ANOVA-Figure 3.10, Figure 3.11
Ca accumulated by roots and shoots of 4 cauliflower cultivars grown in low-Ca or full-Ca nutrient solution
Ca accumulated (quartiary root of mg Ca)

Source	DF	SS	MS	F
V	3	1.2311116	0.4103705	36.99**
T	1	223.0746082	223.0746082	14898.88**
V x T	3	0.4651145	0.1550382	32.25**
H	2	0.4842144	0.2421072	18.36**
V x H	6	0.0552867	0.00921	0.57ns
T x H	2	0.3682720	0.1841360	19.82**
V x T x H	6	0.3154445	0.0525741	6.77**
P	1	6.1358181	6.1358181	114.00**
V x P	3	0.4504452	0.1501484	11.09**
T x P	1	7.2235198	7.2235198	219.08**
V x T x P	3	0.0549333	0.0183111	1.85 ns
H x P	2	6.2805662	3.1402831	204.95**
V x H x P	6	0.1856507	0.0309418	1.50 ns
T x H x P	2	3.7854344	1.8927172	109.78**
V x T x H x P	6	0.1140629	0.0190105	1.11 ns
R	4	0.1674707	0.0418677	
V x R	12	0.1331460	0.0110955	
T x R	4	0.0598903	0.0149726	
V x T x R	12	0.3688177	0.03073481	
H x R	8	0.1054955	0.0131869	
V x H x R	24	0.3895803	0.0162325	
T x H x R	8	0.0743400	0.0092925	

A.16 continued ANOVA-Figure 3.10, Figure 3.11
Ca accumulated by roots and shoots of 4 cauliflower cultivars grown in low-Ca or full-Ca nutrient solution
Ca accumulated (quartiary root of mg Ca)

Source	DF	SS	MS	F
V x T x H x R	24	0.1864614	0.0077692	
R x P	4	0.2152954	0.0538238	
V x R x P	12	0.1625096	0.0135425	
T x R x P	4	0.1318867	0.0329717	
V x T x R x P	12	0.1190824	0.0099235	
H x R x P	8	0.1225760	0.0153220	
V x H x R x P	24	0.4964420	0.0206851	
T x H x R x P	8	0.1379237	0.0172405	
V x T x H x....				
R x P	24	0.4108018	0.0171167	

A.17 ANOVA-Table 3.8
Shoot dry matter produced by 4 cauliflower cultivars grown in low-Ca nutrient solution culture with 4 pH treatments
Shoot dry weight

Source	DF	SS	MS	F
R	4	3.650	0.913	
T	3	5.936	1.979	7.538**
T x R	12	3.150	0.262	
V	3	210.686	70.229	152.731**
V x R	12	7.461	0.622	
T x V x R	48	22.071	0.460	
Total	79	252.955		

A.18 ANOVA-Table 3.9
Root dry matter produced by 4 cauliflower cultivars grown in low-Ca nutrient solution with 4 pH treatments

Root dry weight

Source	DF	SS	MS	F
R	4	0.028	0.007	
T	3	0.065	0.022	3.962*
T x R	12	0.066	0.006	
V	3	2.054	0.685	153.087**
V x R	12	0.069	0.006	
T x V x R	48	0.215	0.004	
Total	79	2.498		

A.19 ANOVA-Table 3.10
Total Ca accumulated by 4 cauliflower cultivars grown in low-Ca nutrient solution culture with 4 pH treatments

Total Ca

Source	DF	SS	MS	F
R	4	14.164	3.541	
T	3	99.042	33.014	20.139**
T x R	12	19.672	1.639	
V	3	695.003	231.668	130.884**
V x R	12	29.054	2.421	1.368ns
T x V x R	48	84.961	1.770	
Total	79	941.896		

A.20 ANOVA-Table 3.11
CaER for 4 cauliflower cultivars grown in low-Ca nutrient solution with 4 pH treatments

CaER

Source	DF	SS	MS	F
R	4	90333.950	22583.487	
T	3	72730.737	24243.579	4.95*
T x R	12	58722.450	4893.538	
V	3	1012432.137	337477.379	54.963**
V x R	12	42529.213	3544.101	
T x V x R	48	294726.400	6140.133	
Total	79	1571474.887		

A.21 ANOVA-Table 3.12
Root length of 5 cauliflower cultivars grown for 10 days in 207 umol Al and 1250 umol Ca

Root length

Source	DF	SS	MS	F
Total	49	375.2648		
Treatment	1	94.1192	94.1192	12.854**
Replication	4	8.7328	2.1832	0.298^{ns}
T x R	4	29.2888	7.3222	
V	4	93.7128	23.4282	8.0695**
V x T	16	78.4288	4.9018	1.6884^{ns}
V x R	16	24.5292	1.5331	0.5281^{ns}
V x T x R	16	46.4532	2.9033	

A.22 ANOVA-Table 4.1
Screening of 2 F1 populations, E x I and I x E, and parent cauliflower cultivars

Total plant weight

Source	DF	SS	MS	F
Total	59	179.4855		
Treatment	3	176.9785	58.9928	1279.671**
Replication	14	0.5725	0.0409	
T x R	42	1.9345	0.0461	

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